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Infections with branching, acid-fast, mycelial organisms of the streptothrix type are sufficiently rare to justify a detailed account of such cases together with a description of the organism. There is perhaps no field in all bacteriology which is more unsatisfactory, so far as classification is concerned, than the study of these higher fungi. The strains appear to be very plastic and hardly any two of them are identical in all details. For many years reports of these infections have been accumulating and at the present time both clinical and bacteriological data are fairly abundant. Much of this information has not been correlated but more recently some important studies have appeared, notably the work of Wright,[1] Musgrave, Clegg and Polk,[2] Foulerton,[3] and especially of Edith Claypole.[4]

Wright has studied exhaustively certain members of this group. He contends that the organisms of the actinomyces type, as found in cattle and in man, are clearly different from other members and maintains that the term, *actinomyces bovis*, first given them by Bollinger should be their proper name. He thinks that the other organisms often classed in this series should be termed after Nocard's work, "nocardia" and the disease "nocardiosis." The nocardia, he says, are "distinguished from the organisms of actinomycosis in that they do not occur in the inflammatory fluids or lesions in man in the compact structures or form the radiate club-bearing structures or granules so characteristic of actinomycoses. Moreover, the organism of actinomycosis does not resist decolorization by dilute acids after having been stained with fuchsin. Localization of the lesions about the mouth and jaw so common in actinomycosis is unknown in nocardiosis." From the point of view of practical medicine there is much to be said

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* Submitted for publication Feb. 5, 1914.

1. Wright: Jour. Med. Research, 1905, xiii, 349; also Osler's Mod. Med., i, 340.
2. Musgrave, Clegg and Polk: Philippine Jour. Sc., 1908, iii, 447.
3. Foulerton: Lancet, London, 1910, i, 551, 626, 771.
4. Claypole, Edith: Jour. Exper. Med., 1913, xvii, 99.

in favor of Wright's view, for, clinically, infections with actinomyces form a well-defined disease which agrees both in animals and in man in many fundamental particulars.

Claypole has attempted to correlate various strains of this group by immunological tests, especially by the complement-fixation phenomenon. She used a large number of strains isolated from various sources including among others several actinomycetes, *Streptothrix madurae, S. asteroides, S. nocardii, S. eppingeri, S. coprae, Bacillus leprae* and *B. tuberculosis*. Many of the organisms studied show more or less of the acid-fast quality but considerable variation in this respect was noted, the *B. leprae* and *B. tuberculosis* being of course most constant. She concludes that it is impossible to draw a sharp line between the various strains from any point of view, morphological, biological, or serological. They form a more or less continuous series and she indicates the change, which is gradual from the mycelial organisms to the bacillary acid-fast organisms, by curves which represent fixation with any given antigen.

I wish to give here the more important details concerning a case of infection with a typical acid-fast mycelial organism which evidently belongs in the streptothrix group and which, according to Wright, should properly be called a nocardia.

History.—The patient is a native-born American, aged 64, single, a teamster. After being acutely ill for three days he was admitted to St. Luke's Hospital, Chicago, in the service of Drs. Favill and Clark, to whom I am indebted for the clinical data. For several years he has been troubled with a chronic cough and at times raises a large amount of thick tenacious sputum. He has been working around horses, but none of the animals so far as he knew had been ill. He has not decreased in weight for several years past. One morning the patient was awakened by a severe, sharp stabbing pain in the right chest. The pain was constantly present but was increased by deep breathing or movements of the body and arms. The patient also had several severe chills later in the day. The pain gradually increased on the following day and severe dyspnea developed, making it impossible for him to lie down. The dyspnea was very severe for a few days and then became less. On the day following the acute onset he began to have a loose cough with expectoration of much white, tenacious, odorless sputum. Excepting the respiratory system no findings of any significance were noted. The following data were obtained at the time he entered the hospital and when he was acutely ill.

Examinations.—There is some hyperemia of the pharynx and uvula and some greenish mucus is adherent to the pharyngeal wall. The chest is well developed; there is a marked depression over the supra- and infraclavicular fossae. Dyspnea is marked and the expansion is deficient, being very much restricted on the right side. The respiration is 28 per minute and is very shallow. There is a frequent difficult cough with expectoration of abundant greenish tenacious sputum. The same conditions of expansion are revealed by palpation as by inspection. Tactile fremitus is much greater over the right chest anteriorly and posteriorly above the level of the angle of the scapula. Below that line on the right side posterior to the axillary line, it is practically absent. The breath sounds are normal over the left lung. On the right posterior side there is an area of flatness over which the sounds are absent or very dis-

tant. Over the area of relative dulness, posteriorly, the sounds are weaker than over the corresponding area of the opposite side and coarse bubbling râles on inspiration are heard over the right lower lobe anteriorly and posteriorly. There is marked pyorrhea. The spleen is not enlarged and the outlines of the stomach and liver are normal. The cervical glands are just palpable but not tender. Other glands are not enlarged.

Two roentgenologic examinations made soon after the patient's admittance showed increased density over one-third of the right lung, chiefly in its lower portion. The plates present the appearance of tuberculosis of the lungs.

The patient's respirations during a period of about ten days ranged between 28 and 44 per minute. Later the number of respirations gradually diminished to about 20 to 25 and then remained constant. The pulse was irregular, ranging from 80 to 130.

The temperature chart of this patient is interesting (Fig. 1.) With remarkable regularity the temperature fluctuates from normal or thereabout in the morning to 101 or 102 in the afternoon. After the first ten days the temperature became decidedly lower; but a few days later the fever was again more marked. After leaving the hospital regular temperatures were not taken, but occasional temperatures indicated a gradual decline and at present, four months after the acute onset, there is no fever.

The leukocytes on admission were 14,500 and four days later were 15,000. Differential count gave polynuclears 86 per cent., small lymphocytes 8 per cent., and large lymphocytes 5 per cent. The results of blood-culture and urine examinations were negative.

The patient left the hospital after about four weeks. Examination at that time showed that the tactile fremitus had returned below the level of the angle of the scapula on the right side. More breath sounds were heard over the area where they were formerly very weak. There were a few coarse dry râles at the end of inspiration over the lower right lobe. Percussion revealed the same dulness except that it was not so pronounced.

On admission to the hospital tubercle bacilli were searched for in the sputum without result, but during the search, an acid-fast, Gram-positive, branching, mycelial growth was found in the sputum. In twelve different sputa examined during the next two weeks these organisms were constantly found. Many pneumococci, a few streptococci and many bacilli, both Gram-positive and Gram-negative, were also present. The cocci were especially numerous during the acute attack but some were always seen, together with the large number of bacilli, after the acute symptoms had subsided.

Since the patient left the hospital he has been under observation and, from time to time, the sputum is examined. He has improved decidedly. He has had no fever or acute symptoms since, but a slight cough still continues and he raises a small amount of mucoid, slightly purulent sputum. Before the patient left the hospital potassium iodid in moderate doses was given for a time and then discontinued. The organisms were not found in the sputum subsequent to this on several examinations, but about three weeks later they reappeared in small numbers. At present they cannot be found in the sputum.

Whether or not the potassium iodid had anything to do with their disappearance is difficult to say, but such a view is suggestive in view of the fact that others[5] have noted good results in these cases following potassium iodid and also in view of the well-known virtues of potassium iodid in other more or less closely related infections, such as sporotrichosis and actinomycosis.

5. Kremers, E. D.: U. S. Army (personal letter).

The streptothrix was very numerous in some specimens of sputum, there being several clumps in nearly every field. In other specimens again, they were difficult to find. In the thick greenish tenacious masses of sputum they were more abundant than in the more mucoid portions. Careful search in all specimens failed to reveal masses that in any way resembled the granules or "sulphur bodies" of actinomycosis. There was never any appreciable odor to the sputum.

Microscopically the organisms appear in small clumps of tangled mycelium with distinct branches (Fig. 2). The filaments are irregular and often at the tip is a bulb-like expansion. No definite ray formations with clubs were ever seen. The mycelial filaments are rather fine but are slightly thicker than a tubercle bacillus. They are Gram-positive throughout. They stain readily with carbolfuchsin and resist decolorizing with acid-alcohol. This resistance is distinctly less than that of tubercle bacilli. The following data were obtained with reference to the alcohol and acid-resisting properties of this particular strain in the sputum. Ninety-five per cent. alcohol for three min-

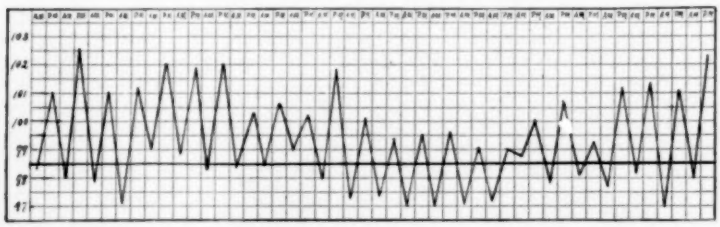

Fig. 1.—Temperature chart of author's patient.

utes does not decolorize them nor does 2 per cent. nitric acid in 95 per cent. alcohol for three minutes. In one minute 10 per cent. nitric acid alcohol does not decolorize but after three minutes they are completely or nearly completely decolorized. Thirty per cent. acid alcohol destains completely in one minute. Aqueous acid solutions decolorize approximately like the alcohol acid mixture.

A point of interest is their failure to resist antiformin. Mixtures of these organisms and tubercle bacilli treated with antiformin clearly showed the presence of the tubercle bacilli and the complete disappearance of the streptothrix. In this respect they resemble the actinomyces types more closely than the tubercle bacillus.

Numerous attempts were made to cultivate these organisms. Sputum containing large numbers in smears were streaked on various mediums, including sugar, blood and ascitic agar. Also plate-cultures and deep tube-cultures were made in various mediums. The cultures

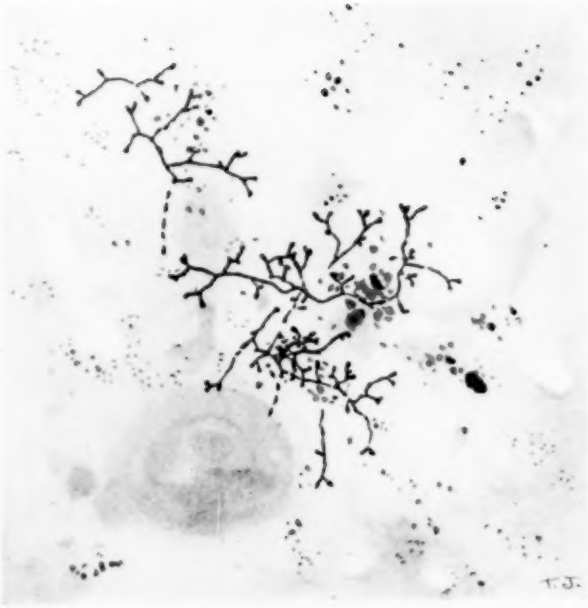

Fig. 2.—Acid-fast streptothrix (Nocordia) appearing in small clumps of tangled mycelium with distinct branches, frequently with a bulb-like expansion at the tips.

Illustrating Article by David John Davis, M.D.
The Archives of Internal Medicine

were carefully observed for weeks and in some instances months for the possible development of the mycelial growths. In no instance was there any growth or multiplication of the streptothrix either under aerobic or anaerobic conditions or at room or incubator temperatures. In this connection mention should be made of the fact that always in the sputum myriads of other germs were present which could have interfered with the development of the streptothrix. There has not been as yet in this case any opportunity to obtain fluids, such as pleural fluid, which might contain the organisms pure. During the acute stage there was some evidence of fluid in the pleural cavity and a few clear drops were obtained by puncture. Unfortunately, this fluid was not preserved for cultures. It is unlikely, however, that any organisms were present, inasmuch as there was no further accumulation later nor were there signs at any time of empyema.

Six guinea-pigs, four young rabbits and a number of white rats were inoculated both subcutaneously and intraperitoneally with moderate and large doses of emulsions of the sputum, which by smear was shown to contain many of the acid-fast organisms. Usually a small induration or an abscess formed at the site of injection, and examination at varying intervals later revealed many bacteria. In no instance did smears stained by the Gram method and by carbolfuchsin show the presence of organisms of the streptothrix type. The lesions all healed after the course of from one to two weeks and in none of the animals did lesions develop that were at all suggestive of this type of infection. It is to be noted that these animal experiments exclude the possibility of these acid-fast organisms being forms of tubercle bacilli.

In the literature on this subject it seems that, while the terms "streptothrix," "cladothrix," "nocardia," "oospora," and "actinomyces" have all been applied to these organisms, the generic term "streptothrix" is being used for this group by most pathologists. J. H. Wright and a few others insist, as pointed out above, that "nocardia" is their proper name because it conforms to the rules of botanical nomenclature. Without going into a close analysis of this matter (which has already been done by several writers), I shall merely say that it seems convenient to use the term "streptothrix" as a generic term, and that it is also convenient to look on these weakly acid-fast mycelial organisms as a subgroup which lies between the organisms of the actinomyces type which are non-acid-fast on the one hand and the strongly acid-fast organisms of the tuberculosis group on the other.

Considerable variation exists among these acid-fast organisms. The one described by Nocard[6] in 1888, as the cause of a disease of cattle known as *farcin du boeuf*, is acid-proof and is distinctly pathogenic for guinea-pigs, producing lesions of a tuberculous character. Eppin-

6. Nocard: Ann. de l'Inst. Pasteur, 1888, ii, 293.

ger[7] was the first to isolate and describe in a human infection an acid-fast streptothrix which he called *Cladothrix asteroides* and which is now usually referred to as *Streptothrix eppingeri*. This case presented pulmonary lesions and a cerebral abscess. Since then about thirty cases have been described by various writers and in various countries. In a number of cases the organisms were not isolated in culture nor were animal experiments successful. In these respects the various strains seem to vary widely from each other. Some of the organisms grow quite readily on artificial culture mediums, as for example *Streptothrix eppingeri*. Others grow with very great difficulty or not at all, as was true of the organisms from the patient here presented, and also of the organisms from Flexner's case,[8] from that of Buchholz,[9] and also from the case recently reported by Steele and Lee.[10] In certain cases in which, from the symptoms and post-mortem examination or from the smear preparations, there can be no reasonable doubt that they were of this type, no attempt was made to cultivate the organisms nor were animal experiments made. This, for example, is true of the report of Butterfield[11] in which case at necropsy was found a well-defined lung lesion in the walls of which were many acid-fast mycelial organisms.

It is a point of interest that nearly all these infections involve the respiratory tract, where they produce lesions which can not be differentiated clinically from pulmonary tuberculosis. In some of them, as was true in this case, acute symptoms with signs of bronchopneumonia or abscess may supervene. Empyema is also a common complication and secondary infections, as might be expected, practically always complicate the course of the disease.

Another point that should be mentioned is the possibility that a small acid-fast fragment or filament might be mistaken for a tubercle bacillus. In the sputum of the preceding case, here and there, could be seen small red filaments which, were they seen alone, might be misinterpreted. In order to differentiate them from the tubercle bacilli the following points should be noted. The filaments are slightly thicker than tubercle bacilli. They are decolorized more readily and by weaker acids than tubercle bacilli. They do not resist antiformin. Furthermore, negative results with guinea-pig inoculations will practically exclude tuberculosis.

Sometimes tubercle bacilli show a considerable degree of branching and it is possible that their identity can only be demonstrated by guinea-pig inoculations. This has been shown by W. H. Smith of

7. Eppinger: Beitr. z. path. Anat. u. z. allg. Path., ix, 276.
8. Flexner: Jour. Exper. Med., 1898, iii, 435.
9. Buchholz: Ztschr. f. Hyg. u. Infectionskrankh., 1897, xxiv, 470.
10. Steele and Lee: Boston Med. and Surg. Jour., 1913, clxix, 502.
11. Butterfield: Jour. Infect. Dis., 1905, ii, 421.

Boston, who reports three cases in which branching, acid-fast bacilli were found and which, in animals, caused typical tuberculosis. Attention, however, should be called to the fact that some of the streptothrix strains may produce in guinea-pigs lesions simulating tuberculosis, so that in such cases one should resort to other confirmatory tests. The differentiation of these organisms from actinomyces has already been stated in the first part of the paper.

The question may very properly be considered whether the acid-fast organism here observed was pathogenic or simply a contaminating saprophyte. With the data at hand the question can perhaps not be definitely decided but I believe it is reasonable to assume that this organism is concerned in some way in the production of the pulmonary lesions observed in this case. It is true that acid-fast organisms of the streptothrix type have been seen in sputum in which the observers considered it a saprophyte; but this is very rare. In nearly all cases in which such organisms have been observed in sputum there is strong evidence that they were concerned in a pathologic process. It is interesting in our case to note that it was in the thick, greenish, highly purulent masses of sputum that the mycelial growths occurred, whereas in the less purulent and more mucoid parts of the sputum they were rare or not present at all. Saprophytic organisms, as a rule, grow fairly readily, whereas these organisms, under the varied conditions of cultivation attempted, did not grow at all. It should be said further that the results of animal inoculation with organisms of this group are not reliable so far as determining the pathogenicity for human beings is concerned. This is well exemplified by the organisms of the actinomyces type which have been far better studied in this respect than the acid-fast mycelial organisms. With them the result of animal inoculations in the lower animals is almost invariably negative in spite of the injection of large doses of organisms recently isolated from active lesions. While positive results have been obtained with certain strains of acid-fast organisms by inoculation of animals, especially guinea-pigs, it is undoubtedly true that many other organisms of this type are, in this respect, similar to the organisms of the actinomyces type.

SUMMARY

There is a fairly well-defined group of infections, the great majority of which are pulmonary, caused by weakly acid-fast branching mycelial growths (nocardia) which do not form ray-like granules, These cases clinically are not ordinarily distinguished from pulmonary tuberculosis. A case is cited above in which the pulmonary lesions were very probably caused by such an organism. For the treatment of such cases potassium iodid should be given in large doses.

THE BLOOD IN INANITION*

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Under the supervision of Dr. Francis G. Benedict, director of the Carnegie Nutrition Laboratories in Boston, Mr. Levanzin of Malta fasted for thirty-one days. The full report of the extensive observations made on him during this experience will appear shortly as a Carnegie publication. I am deeply indebted to Dr. Benedict for his permission to make this preliminary report on the blood which I examined. The report includes a somewhat curtailed analysis of the literature and the bibliography is limited to the references quoted. The details of technic and the correlations with the other findings are omitted.

The blood has frequently been studied during inanition, but an exhaustive search through the literature brought to light only a few records of systematic examinations covering so long a period of fasting in man as the case that forms the basis of this report. More or less authenic accounts of long fasts are rather numerous, beginning with those of the early religious rites as recorded in the Bible and continuing to the present day. They have been undertaken from diverse motives, religious, scientific, suicidal, personal aggrandizement, as a fad, and without motive, the result of diseased body or mind.

Richet[1] gives references to some long fasts in the eighteenth century and earlier. More specific are such cases as the one reported by Maples[2] of the woman evidently catatonic, who died after abstaining from food for fifty-five days; Frazier's[3] patient, who lived fifty-four days without nourishment; the German, reported by Voelkel,[4] who chose starvation as a means of suicide and accomplished his end after twenty-four days; "the fasting woman of Aurangabad" (Brown[5]), who was able by her cunning to give the impression, at least, of having

* Submitted for publication, Jan. 2, 1914.

1. Richet, M. C.: L'inanition chez l'homme, Rev. sc., Par. 1889, series 3, xliv, 106.

2. Maples, J. H.: A Fast of Fifty-five Days, Med. Brief, St. Louis, 1904, xxxii, 905.

3. Frazier, B. C.: Prolonged Starvation, Louisville Month. Jour. Med. and Surg., 1908-9, xv, 147.

4. Voelkel, A.: Freiwilliger Hungertod, Deutsch. med. Wchnschr., 1886, xii, 533.

5. Brown, E. H.: The Fasting Woman of Aurangabad, Med. Rec., Calcutta, 1890, i, 102.

gone without food and drink for years; Merlatti's[6] public fast of fifty days; also the public and well-controlled fast of Dr. Tanner, which lasted for forty days, being absolute for the first fifteen, the report published by Wilder[7]; and A. Jacques' two fasts, the first a private one of forty days and the other a public exhibition lasting thirty days, both reported by Noël Paton and Stockman.[8] These cases, however, are of interest only in establishing some idea as to how long man can exist on his own tissues, as practically no observations were recorded on them, except some chemical data in the instance last referred to. Fasts of man conducted under scientific supervision and including blood examinations are limited in number, though the work on animals has been rather prolific.

Among the earliest references are those to the work of Valentin[9] in 1838, who concluded that there was no alteration in the relation of blood weight to the body weight as a result of exhaustive starvation and that of Bidder and Schmidt[10] in 1852, though the latter do not report anything more specific than an increase in the solid constituents in the blood of a starving cat. With reference still to the blood as a whole, London,[11] much later, studying a series of eight rabbits from which both food and drink were withheld, found a loss in total quantity, proportional, though, to loss of body weight. Paschutin[12] concludes as the result of the work of Heidenhain, Panum and Voit, that the blood is not impoverished by fasting; on the contrary, in certain periods the organism is plethoric. He holds it as remarkable that the number of erythrocytes increases, probably, however, only because of the rapid decrease in plasma. The latter fact is demonstrated markedly in the dogs observed by W. Müller and Buntzen[13] though in none of their animals did a loss of more than 15 per cent. in body weight occur. Luciani[14] holds that aside from water content, the blood exhibits a resistance similar to the nervous system and that the apparent fluctuations, in corpuscular content at least, depend chiefly on the amount

6. Merlatti, Stefano: Histoire d'un jeûne célèbre, etc., Paris, 1887.
7. Wilder, A.: Dr. Tanner and His Famous Fast, Am. Med. Jour., St. Louis, 1896, xxiv, 529.
8. Noël Paton, D., and Stockman, R.: Observations on the Metabolism of Man During Starvation, Proc. Roy. Soc., Edinburgh (1888-9) 1890, xvi, 121.
9. Valentin: Repel. f. Anat. u. Physiol., 1838, iii, 156.
10. Bidder and Schmidt: Die Verdauungsfahrte in der Stoffwechsel, 1852, p. 328.
11. London, E. S.: Note sur la question du changement de la quantité générale et de l'alcalinité du sang dans le jeûne absolu., Arch. des. sc. biol., 1895-96, iv, 516 (abstract by Mühlmann[11]).
12. Paschutin: Pathological Physiology, Inanition, 1902, vol. ii, pt. i, 81 (Russian).
13. Müller, W., and Buntzen: Transfusion and Plethora, Christiania, 1875.
14. Luciani, L.: Das Hungern, Leopold Voss, Hamburg u. Leipzig, 1890.

of water consumed. Chossat (quoted by Paschutin[12]), however, considered that, next to the fat, the blood suffered the greatest loss, amounting even to 75 per cent. of its former weight. This view is not tenable in the light of practically all other work and was evidently the result of faulty observations. Paschutin[12] quotes Valentin as noting striking general changes during hibernation, in part, that the blood putrefies from two to four times more slowly, that the arterial blood is not so bright a red, and that the venous blood is not so dark as normally, because of disturbance of oxygen interchange.

Considering more specifically the blood elements and beginning with the erythrocytes, we find that as early as 1843 Schultz[15] and in 1856 Jones[16] observed atrophic and decomposed cells in starving animals. Others since then have noted these striking alterations in shape and size of the corpuscles, among them Manassein,[17] Andral-Gavarret,[17] Laptschinski,[17] Tanner[17] and especially Kagen,[18] who studied dogs and rabbits. He found little change in the first days, but as the fast progressed the red cells became smaller and crenated ones appeared more frequently until at the end, many "star forms" were seen and microcytes predominated. Liuboumdrow[19] also found variations in the character of the red cells, macrocytes, microcytes and nucleated cells being common, especially the large form which reached 20 per cent. to 30 per cent. of the total red count. Curtis[20] made systematic observations of Griscom's blood during his forty-five-day fast in 1880. This constitutes, by the way, the longest period with examinations of which any record could be found. Curtis describes in great detail marked alterations in shape, size and consistency of the red cells, illustrating not only the painstaking care with which the observations were made, but somewhat the comparative crudity of the methods employed at that time. As will be seen it is only in the earlier reports that the appearance of macrocytes, microcytes, crenated and distorted cells are recorded and it has occurred to the writer that with the improvement of blood technic, the occasion for their presence was eliminated.

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15. Schultz: Beitr. z. phys. u. path. Chemie. von Simon, 1884. (Quoted by Mühlmann[17]).

16. Jones: Smithsonian Contributions to Knowledge, 1856.

17. Mühlmann, M.: Russische Literatur über die Pathologie des Hungerns, Centralbl. f. allg. Path., 1899, x, 160.

18. Kagen, J.: Blood and Blood-Pressure in Starving Organisms, Diss. St. Petersburg, 1884, Russian; from the Laboratory for General and Experimental Pathology, Prof. W. Paschutin, St. Petersburg.

19. Liuboumdrow, P.: Changes in the Blood and Organs in Starvation, Diss., 71, 1893, Russian; from the Laboratory of path. Anat., Prof. W. Winogradow, St. Petersburg.

20. Curtis, Lester: Physiology of Autonutrition; 1. Study of Blood During a Prolonged Fast, Am. Assn. Advanmt. of Science, 1880, xxx, 95.

In 1887 Senator[21] reported finding a number of microcytes on the thirteenth day of a *Schlafsucht* in which a woman fifty-four years old lay for about seven weeks, receiving as nourishment only a small amount of milk and wine. In Succi's[22] blood, late in the forty-day fast made as a public exhibition in London in 1880, numbers of imperfect blood disks were observed. Charteris,[23] on the other hand, could find no alteration in shape, size or staining qualities of the erythrocytes in the blood of his human subject during the fast of fourteen days in 1907. A few nucleated ones did appear during the last four days. Two examinations were made on Dr. Gayer's blood by Dr. Wile.[24] On the eighteenth day the red cells numbered 5,192,000 and on the thirtieth, 5,776,000, a slight rise. The absence of anisocytosis or any degeneration of cells is mentioned specifically in the report.

In none of the other reports are the characteristics of the individual red cell noted, so it is most likely that no alterations of importance occurred.

Continuing the consideration of the effects of inanition on the numerical estimation of the erythrocytes, the results are found to be at rather wide variance.

Senator[21] found practically no change during the long period of almost complete inanition mentioned above. He attributed little value to the small number of counts he made, but concluded there was probably a slight diminution. During both Cetti's eleven-day and Breithaupt's six-day fasts studied by Senator, Lehman and others[25] an increase was noted, amounting, in the former subject to a million. Dupérié[26] also claims that a considerable increase in number occurs. From the study of Succi's blood during a thirty-day fast supervised by Luciani,[14] the latter concludes that the variations noted in numbers are only relative to the concentration or dilution of blood, from differences in water content. Malassez[17] and Lépine[17] found that while in the beginnings of the fasting periods there was an increase in number, in the later days a decrease occurred.

21. Senator, H.: Ueber einen Fall von sog. Schlafsucht mit Inanition, Charité-Annalen, 1887, xii, 316.

22. The Fasting Man, Brit. Med. Jour., 1890, i, 1444.

23. Charteris, Francis J.: Record of Changes Observed in the Blood Count and in the Opsonic Power of a Man Undergoing a Prolonged Fast, Lancet, London, 1907, ii, 685.

24. Dr. Gayer's Fast, a personal communication from Dr. Wile of New York City.

25. Senator, Lehman et al.: Untersuchungen über zwei Hungernden Menschen, Virchow's Arch. f. path. Anat. u. Physiol., 1893, cxxxi, Supplementheft, p. i.

26. Dupérié: Sur les variations physiologiques dans l'état normal des globules du sang, Paris, 1878; cited by Rollett in Hermann's Handbuch der Physiologie, iv, part 1.

Clinical records furnish us with the two following reports. The first is one of Landouzy's patients studied by Malassez.[27] A boy of eighteen lived three months and twenty days with a stricture of the esophagus, the result of swallowing sulphuric acid. He obtained practically no nourishment as he vomited food administered by tube. There was a striking loss, the red cells numbering 3,200,000 the day he died. The second case, reported by Brouardel[28] was a man of forty-eight, who lived four months and twelve days after an experience similar to that of the boy mentioned above. But one blood examination was made, and that two days before death, when the erythrocytes were 4,849,000 and the leukocytes 7,852. While I would regard these as practically normal counts, the author of the report concludes that they demonstrate a concentration of the blood.

Kagen,[18] in 1883, claimed that the ordinary methods of determining the cell content of the blood were open to so many sources of error that the results were not dependable. He limited his observations, therefore, to the direct estimation of the solid constituents, the specific gravity (by pyknometer) and hemoglobin content (Malassez's hemochronometer). He examined six dogs and found in the early days an increase in all three factors, attributing the result to concentration through water loss. The amount of solid constituents, he maintains, can equal even at the end of the fast that present under normal conditions.

Liuboumdrow[19] noted as an average of observations on seventeen dogs a slight increase in erythrocytes until the loss of body weight amounted to from 10 to 15 per cent., then a steady decrease until death, the diminution amounting to as high as 32 per cent. on the twenty-eighth day. Nasse[29] also found an increase in number in a dog after eleven days of complete fasting. As proof that this was due to variation in water content, he states that he obtained a reaction in the opposite direction when the animal was again allowed water.

Poletaëw[30] studied eight dogs that received neither food nor water, dying after loss of 50 per cent. in body weight. These all showed an increase in red cells until late in the fasts, after a loss of 30 per cent. body weight, when there was a gradual decrease till death. Poletaëw is not satisfied with the explanation of this finding simply on the grounds of concentration, for he found an increase also in the dogs

27. Malassez: Bull. et mém. Soc. méd. d. hôp. de Paris, 1874, xi, p. 124.

28. Brouardel: Union Méd., 1876, series 3, xxii, p. 408.

29. De Martigny and Nasse: Ueber den Einfluss der Nahrung auf das Blut, Marburg and Leipsic, 1850.

30. Poletaëw, P.: The Morphologic Composition of the Blood in Complete and Incomplete Starvation in Dogs, Diss. 97, 1894, St. Petersburg (Russian); from the Laboratory of Pathological Anatomy, Professor Uskow.

that were allowed water. He holds that while there may be interference with blood formation, there is also less destruction for bile formation.

Tauszk[31] in a study of Succi's blood during one of his thirty-day fasts, found, after a short interval of decrease, a moderate increase in the red cells. Daiber[32] in 1896 drew his conclusions as to the effect of inanition on the blood, from his findings in Succi's urine during a twenty-day fast. There was an increase in urobilin and earthy alkaline phosphates, both of which to be accounted for by assuming an enormous destruction of erythrocytes, though he does concede that the phosphates might have come from tissue destruction elsewhere. As proof of the adaptability of the blood to altered conditions, he presents the decrease in urobilin and disappearance of phosphate sediment noted after the fifth day, which shows an acquired resistance to the previously destructive influence of fasting. The urobilin was distinctly demonstrable throughout the fast, though greatly reduced, but the phosphates sediment was replaced by urates. The plasma remained intact, as no transudation of its constituents, particularly albumin, through the kidneys could be demonstrated. Daiber concludes that the conditions during inanition must resemble those present in continued fevers in which there is usually red-cell destruction sufficient to give rise to a demonstrable anemia. In these cases, urobilin is present in the urine in distinctive amounts.

Hayem[33] and Reyne[34] each found an increase in erythrocytes in the dog they studied, both animals dying on the twenty-fifth day of fast. Charteris,[23] however, in the case of his already mentioned, could find no suggestive variation, though there was some daily fluctuation.

Gordon,[35] studying the blood of Martin, a medical student who underwent a nine-day fast, could find practically no variation in red-cell count, except that on the sixth day of refeeding, it was about 1,000,000 below the normal. At the end of the first week of Succi's fourth fast, one of forty days conducted in London,[22] the red cells numbered 6,500,000, an increase of 1,000,000 over the average normal individual's count. It may be more or less in this particular case, as the normal count is not given. A. R. Diefendorf, however, found a

31. Tauszk: Jahresb. ü. d. Fortschr. d. Thier-Chem., 1894, xxiv, 147; abstr. from Orvosi hetil., Budapest, 1894, p. 512.

32. Daiber: Beitrag zur Kenntnis des Stoffwechsels beim Hungern, Schweitzer Wchnschr. f. Chem. u. Pharm., 1896, No. 42, xxxiv, 395.

33. Hayem, G.: Leçons sur les modifications du sang, Paris, 1882, p. 382.

34. Reyne: Quoted by E. Bardier in his article on Inanition in Dictionaire de Physiologie, Charles Richet, ix, 99.

35. Gordon, Martin A. A.: A Prolonged Fast, Montreal Med. Jour., 1907, xxxvi, 482.

slight diminution during and a relatively rapid rise immediately following the two fasts of seven and four days, interrupted by a feeding period of nineteen days, which formed the basis of Benedict's[36] report.

Three counts were made on the blood of Dr. Penny,[37] who fasted for thirty days, in 1909, drinking only distilled water. They demonstrated a moderate increase till the twentieth day and a loss of 1,000,000 during the remaining ten days. Here again no normal count was obtained. Ronsse and van Wilder[38] hold that there will always be a slow increase in erythrocytes if water as well as food is withheld.

Though the conditions are not altogether analogous, it is interesting to note that in hibernating animals there is a decided decrease in erythrocytes, as is reported by Ranke.[39]

HEMOGLOBIN

Senator, using the von Fleischl method, noted a moderate increase in his woman subject[21] and in Breithaupt;[25] a loss, however, of about 20 per cent. in Cetti[25] in nine days; Liuboumdrow[19] (with Malassez's method) found a slight increase in the blood of dogs until a loss of from 10 to 15 per cent. of body weight had occurred, when a decrease was recorded, that progressed until the animals died; Benedict[36] reports a slight loss during the fasts, with a rise afterward, corresponding to the fluctuations of the erythrocytes. The von Fleischl and Tallqvist methods were used. Martin's[35] hemoglobin was 90 per cent. the first day, 95 per cent. the fourth and 90 per cent. again on the last day, the 9th, dropping to 80 per cent., six days after resuming food. Penny[37] showed an increase of 8 per cent. during his thirty-day fast, going from 104 to 112 per cent. Luciani[14] concludes that there is an actual loss of hemoglobin. Quoting his studies with Bufalini carried out in Sienna in 1882 on a dog that lived fifty-three days without food, he states there was a rapid rise during the first six days (modified Bizzozero's method used). This he explains as being due not only to concentration from loss of water but also to the more rapid consumption of plasma than corpuscles.

Gayer's[24] percentage rose from 80 on the eighteenth day to 100 on the thirtieth, when his fast was broken. Charteris[23] reporting the fourteen-day fast, notes a drop from 110 per cent. to 96 per cent. after

36. Benedict, Francis G.: The Influence of Inanition on Metabolism, Carnegie Publication, No. 77, 1907.

37. Penny, F.: Notes on a Thirty-Day Fast, Brit. Med. Jour., 1909, i, 1414.

38. Ronsse, I., and van Wilder, H.: Variations du nombre des globules rouges et du taux de l'hémoglobine au cours de l'inanition chez le lapin, Arch. internat. de pharm. et thér., 1903, xl, 301.

39. Ranke, Johannes: Grundzüge der Physiologie des Menschen, Ed. 3, p. 380.

remaining unaffected for the first few days. The loss was not recovered until several days after breaking fast. Subbotin[40] using Preyer's method (spectroscope) found a decrease of hemoglobin in a patient fed on nitrogen-free diet. By the 26th day it had fallen from 13.80 per cent. to 11.65 per cent. and on the thirty-eighth day it was 9.52 per cent. In starving rabbits, however, there was an increase, due, he concludes, to decrease in water content of the blood. In a dog that starved for thirty-eight days, there was very little variation, the range being from 13.80 at the beginning to 13.33 per cent. at the end of the fast.

Hermann[42] in discussing the subject says that the changes found with the ordinary methods of examination may well be due to concentration, because the water is the most variable of the blood constituents. Results are conclusive, therefore, only when they deal less with the hemoglobin content than with the relation of hemoglobin to the total quantity of solid constituents. Groll's[43] work was carried out on this line in rabbits, cats and one dog. The simple hemoglobin percentage showed a rise in all the animals, being, however, only a relative increase, due to concentration of the blood, while there was no actual diminution in the total solids. He concludes that the hemoglobin is more stabile in starving conditions than any of the other constituents.

It would seem from the preceding data that the red cells and hemoglobin are particularly resistant, though in the long fasts there is no doubt a slight loss. The general consensus of opinion appears to be that concentration of the blood through water loss is responsible for the increase found.

LEUKOCYTES

More attention has been paid the white cells than any of the other blood constituents and reports are more at variance as to just what does happen to them during states of inanition. Almost every possible change, especially numerical, has been observed at one time or another. Morphologic alterations are recorded by Luciani.[14] He noted a decrease in size that appeared early, so that by the fifth day all the leukocytes were smaller than the red cells. They recovered their normal size by the ninth day, however. Charteris,[23] on the other hand, mentions specifically that he observed no alteration in size. Manasseïn[17] reports the presence in the leukocytes of fasting rabbits of refractile bodies that are not affected by acetic acid. Källmark[44]

40. Subbotin: Ztschr. f. Biol., vii, 187.

42. Hermann, L.: Untersuchungen über den Haemoglobin-Gehalt des Blutes bei vollständiger Inanition, Arch. f. d. ges. Physiol. (Pflüger's), 1888, xliii, 239.

43. Groll, S.: Untersuchungen über den Haemoglobin-Gehalt des Blutes bei vollständiger Inanition; Diss. 1887, Königsberg i. Pr.

44. Källmark, F.: Zur Kenntnis des Verhaltens der weissen Blutkörperchen bei Inanition, Folia Haemat., 1911, xi, pt. i, 411.

observed in his rabbits rarifications in the basophils with agglutination and peripheral arrangement of the granules.

Curtis[20] observed peculiar bodies, resembling leukocytes but larger, consisting of spherules too small to measure. These cells measured 1/2000 inch in diameter and exhibited ameboid movement. When these very indefinite bodies were most abundant, the granules were absent from the leukocytes. Curtis does not speculate as to whether these were altered white corpuscles or a foreign cell entering the blood from the tissues. Hayem[23] concludes that there is no essential change in the leukocytes during starvation, at least in dogs.

Considered numerically as to both total and differential estimations, the following results have been obtained from studies of fasts in man. In both Cetti and Breithaupt[26] a moderate decrease was observed during, with a considerable rise the first few days following fast. Senator[45] concludes that there is a lively new formation of leukocytes on refeeding. Luciani[14] records a marked diminution in the early period of Succi's thirty-day fast, dropping from 14,536, the count on the first day, to 861 on the seventh. The count then rose to 1,550, where it remained with slight fluctuations due to concentration and dilution of blood until the twenty-ninth day. He attributes the marked diminution to the digestive action of trypsin, which evidently enters the blood as such during the cessation of intestinal digestion. He bases this theory on the work of Albertoni, who by intravenous injection of trypsin secured almost complete disappearance of leukocytes. The trypsin apparently has no effect on the erythrocytes. It is quite possible, also, that there may exist in the early days of the hunger period some special destructive condition, evidenced also by the loss of hemoglobin. Two other factors at work, he argues, are, first, the disappearance of the lymphocytes, they no longer being required to alter the assimilated products of digestion in the blood plasma (after the work of Schaeffer, Hofmeister and Zawarykins); secondly, the leukocytes may have lost their *Wanderlust*. There would then not only be a failure of "outwandering" from the blood, but of "inwandering" from the tissues as well and the latter factor would exert the greater influence on the number, because there would be less active replacement of the cells lost by death. The white cells practically disappeared from Succi's blood during his fourth fast, until late, when small and ill-formed corpuscles were found. Tauszk[31] noted a decrease in total count during one of Succi's thirty-day fasts. This was due to loss of mononuclear cells, including lymphocytes. The

45. Senator: Bericht über die Ergebnisse des auf Cetti ausgeführten Hungersversucht, Berl. klin. Wchnschr., 1887, xxiv, 427.

eosinophils and polymorphonuclears were increased. There was little change in the white cell content of Martin's[35] blood during his nine-day fast, except that on the second and ninth days they rose to 10,000. There was a very slight progressive loss in the polymorphonuclears, while the lymphocytes were increased somewhat on the sixth and ninth days. There was no differential count made on the second day to explain the total rise on that day.

The result of the two examinations of Gayer's[24] blood are given in Table 1. The striking points in this case are the very low total count on both occasions, the increase in the small and the drop in the large lymphocytes at the end of the fast. No explanations are offered by Wile.

TABLE 1.—EXAMINATION OF GAYER'S BLOOD

Day	Total Whites No.	Poly. Pct.	L. Lymph. Pct.	S. Lymph. Pct.	Eosin. Pct.	Baso. Pct.
18	2,600	54	12	30.4	1.6	2.0
30	2,800	51	5.4	42	1.0	0.6

In Dr. Benedict's[36] subject, during the first fast of seven days, there was a progressive diminution until the last day, when there was a slight rise. After an interval of nineteen days, there was a second fast of four days. During this period, a gradual rise, amounting to about 2,000, was noted. The polymorphonuclears averaged high during both fasts, but at no time could the number be considered distinctly pathological. The small lymphocytes averaged low. The large lymphocytes were high during the last two days of the first period and throughout the last. The eosinophils were low and the basophils high in both fasts. Charteris[28] in 1907, found a moderate leukocytosis, reaching 14,000 on the sixth day from 5,300, the count before the fast. He noted further a gradual increase of the eosinophils to 7 per cent., a condition that had never been noted before in human blood during inanition. His subject went fourteen days without food, receiving a constant quantity of water, 1 liter a day. Penny's[37] blood showed also a slight leukocytosis with a return to normal at the end. The noteworthy features of the differential counts are the high polymorphonuclear percentage, the very marked falling off of the lymphocytes and the increase in the large mononuclears. Reyne[34] could demonstrate no influence on the leukocytes in his dog that fasted for 25 days. Howe and Hawk[46] studied two men during seven-day fasting periods with uniform water allowance. There was an increase in the polymorphonuclears at the beginning followed by

46. Howe, P. E., and Hawke, P. B.: Fasting Studies; ix. On the Differential Leukocyte Count during Prolonged Fasting. Am. Jour. Physiol., 1912, xxx, 174.

a decrease to below normal by the end of the fast. The small lymphocytes presented the reverse picture, while the large lymphocytes increased during the early days. One subject showed a moderate increase in eosinophils. The blood of both men returned to normal after several days of diet.

Even in animals where conditions can be comparatively readily controlled, there has been a striking lack of harmony in the findings. Rabbits and dogs have been the animals of most frequent choice and Okintschitz's[47] report of his work on the leukocytes of the former in 1892, is one of the earliest. He was concerned only with the differential variations. In normal rabbits the eosinophils constitute about 50 per cent., lymphocytes 25 per cent., large round cells and polymorphonuclears each 12.5 per cent. The animals were allowed no water. He found diminution of the lymphocytes and polymorphonuclears and an increase in eosinophils and large round cells. The polymorphonuclears show the most marked diminution and on refeeding they are increased, seeming, therefore to be the form most affected by food. The disturbance in blood-picture is still evident even when the animals have almost completely regained their body weight. Hayem,[33] much earlier, in 1882, could see no difference between the variations of the leukocytes in the dog studied by him during its twenty-five-day fast and those which occur under normal circumstances.

Argaud and Billard[48] found about the same alterations in the blood of the two rabbits they studied as did Okintschitz. They report a marked hypoleukocytosis with an inversion of the formula, there being present three mononuclears to every polymorphonuclear. The recovery in these animals, however, was more rapid, for in a few days the blood-picture had resumed the normal. Källmark[44] also studied rabbits during periods of complete starvation varying from seven to fourteen days. He noted a primary fall in lymphocytes and polyneutrophils, followed by a rise until the seventh day, slow in the latter form, more rapid in the lymphocytes. In the longer fasts this rise was followed by fluctuations, none of which, however, went as high as the normal counts. The basophils showed a marked rise on the third day of the longer fasts. On refeeding, the polymorphonuclears showed a more rapid rise than the lymphocytes, duplicating the experience of Okintschitz. Källmark concludes that the lymphocytes are supplied in greater abundance during inanition, most prob-

47. Okintschitz, E.: Ueber die Zahlenverhältnisse verschiedener Arten weisser Blutkörperchen bei vollständiger Inanition und bei nachträglicher Auffüterung (Versuche an Kaninchen) Arch. f. exper. Path. u. Pharmakol., 1892-1893, xxxi, 383.

48. Argaud, R., and Billard, G.: Inversion de la formule leucocytaire sous l'influence de l'inanition, Mém. Soc. de biol., 1911, lxx, 746.

ably by the thymus, which in so doing atrophies. The primary fall and the postinanition rise in the leukocytes occur, he believes, before compensation for the disturbances of equillibrium has been established or the organism has adapted itself to the altered conditions. When this has been accomplished, the changes in the blood are not so much different from those noted under normal circumstances.

Rieder[49] reports finding a marked hypoleukocytosis in the dogs he studied in 1892. Liuboumdrow[19] found that the leukocytes of his fifteen dogs decreased gradually at the beginning of their fasts until a loss of 20 per cent. body weight was reached. A gradual rise was then noticed, except in six of them, frequently reaching normal. The lymphocytes showed a diminution persisting to the end, most marked early, dropping from 15 per cent. to 3 per cent. or less. The monocytes reappeared to a certain extent, after the primary fall. The polymorphonuclears were proportional throughout to the total count. Eosinophils appeared early in those animals that did not show them before fasting, and in most cases there was an increase of seven or eight times, which lasted until a loss of from 10 to 30 per cent. in body weight had occurred, when they began to diminish. By the end of the fasts, they had disappeared altogether.

Poletaëw[30] and Reyne[34] both observed great variations in the number of white cells in the dogs they studied. The former concluding that there was evidently a diminution in all the forms until a loss of from 30 per cent. to 40 per cent. in weight and then an increase toward the end, of the younger elements, including lymphocytes. Uskow[30] interprets these results thus: In the beginning, the entrance of young leukocytes into the blood is retarded, as is also the transition of the young into ripe forms. In the latter period, however, the lymph tissue, probably stimulated into increased activity by the products of degeneration, sends more cells into the blood and further, there is probably a more rapid development of the young forms already present into ripe cells. Keuthe[50] noted a decrease in polymorphonuclears and an increase in lymphocytes during the first days and a reversal of this relation in the latter days of fasting. Paschutin,[12] on the other hand, concludes that the fast has practically no effect on the leukocytes, that they show very little alteration.

Howe and Hawke[46] observed the following changes in four dogs receiving only a constant quantity of water: Three of them, going 117, 15 and 30 days respectively, showed a decrease in polymorpho-

49. Rieder: Beiträge zur Kenntnis der Leukocytose, Leipsic, 1892.

50. Keuthe, W.: Ueber die funktionelle Bedeutung der Leukocyten im zirkulierenden Blute bei verschiedener Ernährung, Deutsch. med. Wchnschr., 1907, xxxiii, 588.

nuclears with an increase in the small lymphocytes. The basophils, eosinophils and transitional forms showed no noteworthy changes. The fourth dog, fasting for forty-eight days, was already anemic. His blood presented the reverse picture, the polymorphonuclears increased, the lymphocytes decreased. An eosinophilia that developed early disappeared. Two of these four animals showed an increase in large lymphocytes while the other two a fairly constant decrease in the same variety of cell. During repeated periods of fifteen and thirty days fasting in these dogs, the results were quite different, all the forms remaining practically constant, save the large lymphocytes.

With regard to the question of hibernating animals throwing some light on the findings during simple starvation, the work of Hansemann[51] is interesting. When the animals were killed during their hibernating state and the various organs examined, no evidence of cell division could be found. He concludes that the physiologic cell division occurs as a result of the mechanical wearing out of the tissue. If this is eliminated, as it is under these circumstances when muscular and digestive activity and the general vital processes are practically in abeyance, there is no stimulus for cell division. The reduced activity, the absence of intestinal digestive processes or products and the presence of perverted products of parenteral digestion during fasting, no doubt would be the important factors in influencing the blood picture. Argaud and Billard[48] examined the blood in hibernating dormice and only a few monocytes were found after careful search, the other forms having apparently entirely disappeared. Valentin, quoted by Paschutin,[12] had the same experience, finding only a few white cells. He explains their absence to the lack of lymph, which, he asserts, introduces the leukocytes into the blood.

Interesting also in view of the findings in some of the cases of inanition quoted above are the changes noted in bone marrow by Roger and Josué.[52] In rabbits that were completely starved for six or seven days, the marrow showed the presence of many giant cells. Neutrophilic granular myelocytes predominated, though there were many polymorphonuclear cells. Eosinophilic cells were rare. The fat was largely replaced by a granular, albuminoid substance, not mucin. On refeeding, the eosinophils were even less in evidence, but there were many very large giant cells and numbers of nucleated red cells, some of them polynuclear. Not before twenty-four days of feeding did the marrow return to its normal state. This picture is

51. Hansemann, D.: Ueber den Einfluss des Winterschlafes auf die Zellteilung, Arch. f. Physiol., 1898, v and vi, 262.

52. Roger, J., and Josué: Des modifications histologiques de la moelle osseuse dans l'inanition, Compt. rend. Soc. biol., 1900, lii, 417.

not altogether consistent with the blood-findings reported, especially the eosinophilia described by Okintschitz[47] and the scarcity of polymorphonuclears observed by Argand and Billard.[48]

As already stated, the thymus has been found to atrophy during inanition. Källmark[44] noticed diminution not only in the size of the organ, but in the number of mitotic figures, an evidence of its inactivity. He quotes von Friedleben as having made the same observations as early as 1858, Hammar in 1905 and von Jonson in 1909. These are the only references that could be found to the histological appearances of the hematopoietic organs during starvation. This is a neglected feature of the subject that would seem to offer a profitable field for investigation.

PHYSICOCHEMICAL CHANGES

Specific Gravity

The question of influence of food and drink and the abstinence from them on the density of the blood has been rather frequently the subject of investigation. We find that as early as 1834, Thacrah[53] noted an increase in the specific gravity during hunger periods. J. Davy[54] obtained the same result by depriving his subject only of water and Nasse,[29] starving dogs but allowing water, found a decrease in specific gravity after three or four days, but that by the eleventh day, the blood had returned to or even exceeded its normal density.

Liuboumdrow,[10] using the pyknometer, detected fluctuations in density as marked, comparatively, as those noted in the number of red cells, but a complete agreement between specific gravity and erythrocyte count was not found. Castellino,[55] studying starving rabbits, found an increase in density and at the same time a decrease in the serum content of their bloods. Popel[56] also reports an increase, though a slight one. He studied both rabbits and dogs, using Hammerschlag's method. In the former the increase did not exceed 1.6 per cent. and it was still less in the dogs. After ligation of the ureters, there was the slight fall of 9.11 per cent. from normal in rabbits, while the dogs showed a rise of 0.72 per cent., a rather unexpected result, if taking only the water content of the blood into consideration. London's[11] findings do not agree with those above. He also used Hammerschlag's

53. Thacrah: An Inquiry into the Nature and Properties of the Blood as Existent in Health and Disease, London, 1819-1834.

54. Davy, J.: Physiological and Anatomical Researches, London, 1839.

55. Castellino, P.: La suscettibilitá infettiva nella inanizione lenta, Riv. d'ig. e san. Pub. 1893, iv, 461.

56. Popel, W.: Sur les variations de la densite du sang dans le jeûne absolu, simple, ou compliqué de la ligature des uretères; from the laboratory of general pathology, Prof. S. Lukjanow, Arch. d. sc. biol., 1895-6, iv, 354.

method, but reports a slight diminution in the rabbits that starved for from five to fourteen days, the average dropping from 1.048 to 1.043. The animals in both the above-mentioned series were deprived of water.

There was evidently considerable fall in Martin's[35] blood, for while no preliminary estimation was made, on the sixth day of his fast the specific gravity was 1.026; on the eighth there was a rise to 1.031 and on the ninth and last day it had dropped to 1.021. One week after breaking fast it was 1.043, still very low if we consider the normal to be 1.059 to 1.060. Lloyd Jones is quoted by Lyonnet[57] as finding, on the tenth day of one of Succi's fasts, a specific gravity of 1.061 that rose to 1.063 the thirty-ninth day.

Lyonnet,[57] speaking of the influence of food and drink on the specific gravity, holds that there is usually though not invariably a diminution after the intake of water, the change being but very temporary. Abstinence from all liquid causes an increase that is not so marked, however, as one would suppose. (In this he is quoting Lichtheim). Food apparently has some effect, in that after meals there is a decrease to be found that lasts for an hour or so.

Coagulability

Very little mention is made in the literature of the influence of inanition on the coagulation time. Arnold[17] and Collard de Martigny,[17] both noticed that the clot was larger than usual in relation to the amount of serum and the latter in 1850 found a decrease in fibrin content. Jones[17] also noted that the water and fibrin decreased more rapidly than the solid constituents. Källmark[44] noticed that in rabbits, after the fifth or sixth day of starvation, the blood coagulated more rapidly, but no estimation of the time is given. Vierordt[58] was among the first to refer to this feature of the subject, having made the observation in 1878 that an acceleration of the process occurred as a result of starving. Tria[59] reports quite recently that he could detect very little variation during short fasts in rabbits and dogs. Valentin[9] noticed a marked retardation of coagulation in hibernating animals.

No report of the specific estimation of coagulation time during inanition in man could be found. Dr. Wile[24] reports that on both examinations of Gayer's blood made on the eighteenth and thirtieth days there was apparent decrease in the platelets, but that coagula-

57. Lyonnet, B.: De la densité du sang, sa détermination clinique, ses variations physiologiques et pathologiques, Paris, 1892, p. 73.

58. Vierordt, H. C.: Arch. d. Heilk., 1878, xiv, 193.

59. Tria, P.: Propriétés chimico-physiques du sang durant l'inanition, archiv. ital. de biol., 1911, lv, 49.

tion was accelerated. He says of the last examination that "the blood was thick, dark red and did not flow easily." Aside from such general conclusions without data to demonstrate them, the only clue as to what might be expected in man are a few observations that have been made relative to meal times. Coleman[60] found the longest coagulation time one hour after the principal meal and the shortest before breakfast. Cohen,[61] using the method devised by himself determined that the average time before meals was 7 1/6 minutes and after meals 9 minutes, while Mercier, quoted by Cohen in the above-mentioned article, constantly found the coagulation more rapid after meals than before and Addis[62] asserts that food has no influence on the process. Cohen[61] quotes A. E. Wright as crediting fluids with a greater influence on coagulability of the blood than food, but that hunger does retard the process, a view not upheld by the observations of Coleman and Cohen. Increased consumption of liquids lengthens the time and withholding them has the opposite effect.

Immunity

There have been a few studies made of the effect of starvation on immunity in general and the immune body content of the blood specifically, but the data are scarcely sufficient to warrant definite conclusions. In 1890 Canalis and Morpurgo[63] studied the effect on the natural immunity which pigeons exhibit toward anthrax. They were found constantly to lose this resistance if the fast were begun immediately after the injection of the organisms, or a day or so before. They regained it, however, on refeeding if the inanition period had not been too long. The same natural immunity possessed by chickens[64] was not lost unless they were starved for more than 8 days. If starved before inoculation, they proved more susceptible. These workers were unable to make rats susceptible to anthrax by starving. P. Castellino[55] concluded from his studies on rabbits in 1893 that there was a diminution in resistance to infection. A few years later, 1899, Meltzer and Norris[65] could demonstrate no difference in the bactericidal action against the typhoid bacillus of the blood of starved, under

60. Coleman, C. J.: The Coagulation of the Blood and the Effects of Certain Drugs Upon It, Bio-Chem. Jour., 1906-7, ii, 184.

61. Cohen, M. S.: Coagulation Time of the Blood as Affected by Various Conditions, ARCH. INT. MED., 1911, viii, 684.

62. Addis, T.: The Coagulation Time of the Blood in Man, Quart. Jour. Exper. Physiol., 1908, i, 305.

63. Canalis, P., and Morpurgo, B.: Ueber den Einfluss des Hungers auf die Empfänglichkeit für Infectionskrankheiten, Fortschr. d. Med., 1890, viii, 693.

64. Canalis, P., and Morpurgo, B.: Fortschr. d. Med., 1890, viii, 729.

65. Meltzer, S. J., and Norris, C.: On the Influence of Fasting upon the Bactericidal Action of the Blood, Jour. Exper. Med., 1899, iv, 131.

or overfed dogs. Roger and Josué,[66] on the other hand, having observed an increase in the resistance to the colon bacillus in fasting rabbits suggest that some possible benefit may be derived from fasts. This is the only bit of experimental evidence speaking for the value or advisability of this procedure.

Charteris[23] noticed a wide daily variation in the opsonic index of the blood of his human subject during the latter's fourteen-day fast, but as he obtained a similar result with his own blood, he was led to conclude that the changes during fasting were not significant, due, rather, to the use each day of a fresh emulsion of bacteria. Martin's[35] blood, however, showed a gradual lowering of the index, returning to normal four days after the fast was broken. In this case the *Staphylococcus aureus* was used.

Among the studies on other properties of the blood are to be mentioned those of Tria[59] with reference to the viscosity and electroconductivity in rabbits and dogs. He found little alteration, some decrease in both early. He concludes from his entire study that the body is able to compensate pretty well for the disturbances in nutrition, thus permitting long fasts without real serious consequences. A decrease in alkalescence was noticed by Tauszk[31] in Succi's blood and Benedict reports the same change in his subject.[36] A very moderate decrease was also observed by London[11] in his eight rabbits.

THE AUTHOR'S OBSERVATIONS ON LEVANZIN'S BLOOD

The examinations were concerned principally with the red and white cell and hemoglobin content. There was an attempt made to determine the opsonic index, but the subject was so far from an incubator and centrifuge that it was found impossible to obtain anything like accurate results, so this feature was abandoned, though still recognized as one of the most important lessons to be learned from the blood. The coagulation-time and specific gravity were also investigated.

For three days just preceding the fast, Levanzin's blood was examined to determine a normal picture with which to compare the results later obtained. During the fast, with the exception of the first day and three days scattered through the period, daily examinations were made and in addition, on the first and third day of refeeding. The time of the day did not vary more than half an hour throughout, all the specimens being obtained between 10 and 10:30 a. m., so that there was a constant relation to the general routine of the subject's daily activities, that is, immediately after he had finished

66. Roger, J., and Josué: Influence de l'inanition sur la resistance à l'infection colibacillaire, Compt. rend. Soc. biol., 1900, lii, 696.

with the respirometer experiment, been weighed, washed his face and hands and climbed the short flight of stairs to his balcony. It was his habit to take about half a glass of water before submitting to the lancet-prick. It is well to mention here that after the first three days, the quantity of water ingested daily was uniformly 750 c.c.

The Thoma-Zeiss apparatus was used for counting the red and white cells and smears for the differential counts were stained by the Wright method. For the estimation of the hemoglobin percentage, the Tallqvist[68] and Sahli apparatuses were used.

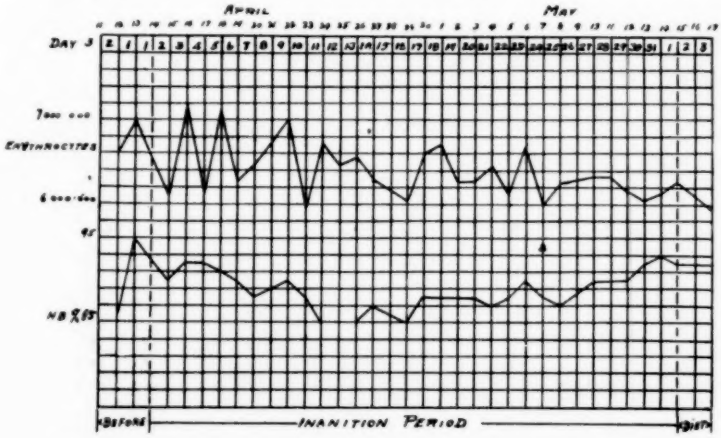

Fig. 1.—Relation of hemoglobin to red cells in fasting subject.

The results of these examinations are contained in Table 2. They are correlated and presented more graphically by Figure 1, to show the relation of hemoglobin to red cells, and by Figure 3, to show the relation of the total white count to the differential. To avoid confusion of curves, the transitionals, eosinophils, basophils are plotted separately in Figure 4 and the scale enlarged. In Figure 2 the composite curve of the polynuclears, that is, neutrophils, basophils and eosinophils and composite curve of the mononuclear cells, large and small lymphocytes, transitionals and monocytes are presented for comparison.

Erythrocyte.—The subject's normal count apparently was high. The three preliminary estimates range well above 6,000,000. It maintained this high figure throughout the test, going below only on two occasions, the tenth day of the fast and the third day following. In

68. Tallqvist, T. W.: Ueber die Anwendung des Filtrirpapiers im Dienst der praktischen Haematologie, Berl. klin. Wchnschr., 1904, xli, 926.

TABLE 2.—Daily Protocol of Blood-Count in Fasting Subject

Day	Hb. Pct.	Erythrocyte	Leukocyte	Poly. Neut.	S. Lymph.	L. Lymph.	Monocyte	Transitional	Eosin.	Basop.
BEFORE FASTING PERIOD										
3	85	8,984,000	5,900	67.5	23.5	2.5	4.5	1.5	0.5	0.0
2	85	6,632,000	6,400	66.0	22.0	1.5	7.5	2.0	0.5	0.5
1	95	7,000,000	6,000	59.5	32.0	3.0	4.0	0.0	1.5	0.0
FASTING PERIOD										
2	90	6,100,000	8,400	73.0	20.0	3.0	3.0	0.5	0.5	0.0
3	92	7,200,000	12,400	79.0	16.5	0.5	3.0	0.5	0.5	0.0
4	92	6,120,000	8,400	66.0	24.0	4.0	4.0	1.0	0.5	0.0
5	91	7,170,000	9,400	68.0	26.5	2.0	3.0	0.5	0.0	0.0
6	90	6,250,000	8,600	67.5	19.0	4.5	3.0	5.0	0.5	0.5
7	88	6,450,000	7,000	60.0	24.0	4.0	3.5	6.5	2.0	0.0
9	90	7,000,000	8,600	66.0	26.0	3.5	1.0	3.0	0.5	0.0
10	88	5,950,000	7,000	68.5	22.0	3.0	3.0	1.5	1.5	0.5
11	85	6,750,000	8,400	68.0	23.0	2.0	5.0	2.0	0.0	0.0
12	85	6,480,000	7,000	74.0	15.0	2.0	4.5	3.0	1.5	0.0
13	85	6,580,000	6,800	64.0	24.5	7.5	1.0	2.0	0.5	0.5
14	87	6,280,000	7,900	64.0	26.5	4.0	2.5	2.5	0.5	0.0
16	85	6,010,000	9,000	72.5	16.5	3.5	1.5	5.0	0.5	0.5
17	88	6,600,000	6,200	64.5	29.0	3.0	0.5	3.0	0.0	0.0
18	88	6,700,000	6,600	65.5	24.0	6.5	2.0	1.0	1.0	0.0
19	88	6,250,000	6,200	62.5	22.5	5.5	3.5	5.0	0.5	0.5
20	88	6,250,000	6,400	61.0	28.5	3.0	3.5	2.5	1.0	0.5
21	87	6,450,000	6,400	62.5	20.5	5.0	5.5	5.5	1.5	0.0
22	88	6,130,000	6,100	61.0	26.5	3.5	6.5	2.5	0.0	0.0
23	90	6,630,000	6,600	58.5	28.0	4.5	4.5	3.0	0.5	1.0
24	88	6,000,000	6,900	65.0	25.0	2.5	4.5	2.5	0.5	0.0
25	87	6,250,000	6,500	65.5	27.0	1.5	5.0	1.0	0.0	0.0
27	90	6,240,000	5,800	63.0	26.0	3.0	6.5	1.5	0.0	0.0
28	..	6,350,000	7,800	61.5	30.0	1.0	5.5	2.0	0.0	0.0
29	90	6,190,000	6,000	63.5	27.5	1.5	5.0	2.0	0.0	0.5
30	92	6,050,000	6,000	64.5	26.5	2.0	5.0	1.5	0.5	0.0
31	93	6,170,000	8,000	60.0	32.0	2.5	5.0	0.5	0.0	0.0
DIET										
1	92	6,280,000	7,200	70.0	21.5	2.5	4.5	1.5	0.0	0.0
2*										
3	92	5,960,000	6,600	60.5	32.5	3.0	3.0	0.0	0.5	0.0

* Too ill for examination.

the early part of the fast there was daily variation, ranging under 1,000,000. This became less evident toward the end. The general impression given by the curve is that of a very moderate decrease. there were no alterations in the characteristics of the individual cells as to size and shape and no nucleated reds were found at any time.

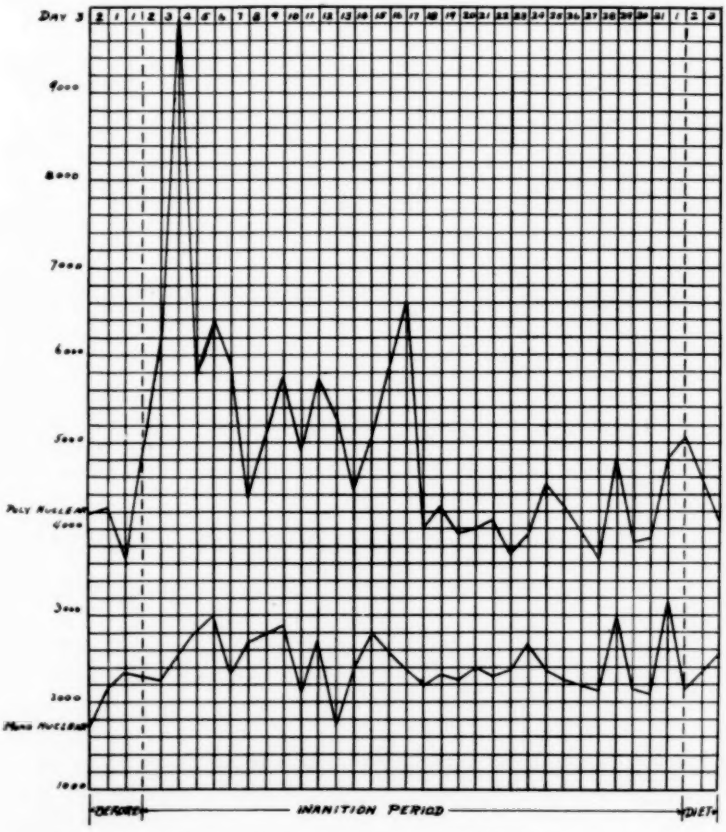

Fig. 2.—Composite curve of the polynuclears (neutrophils, basophils and eosinophils) presented for comparison with a composite curve of the mononuclears, large and small lymphocytes, transitionals and monocytes.

Hemoglobin.—The hemoglobin average ranges rather consistently above 85 per cent., the most marked variations being consistent with those of the erythrocytes. The low period is between the tenth and sixteenth days, from then on showing a very moderate rise.

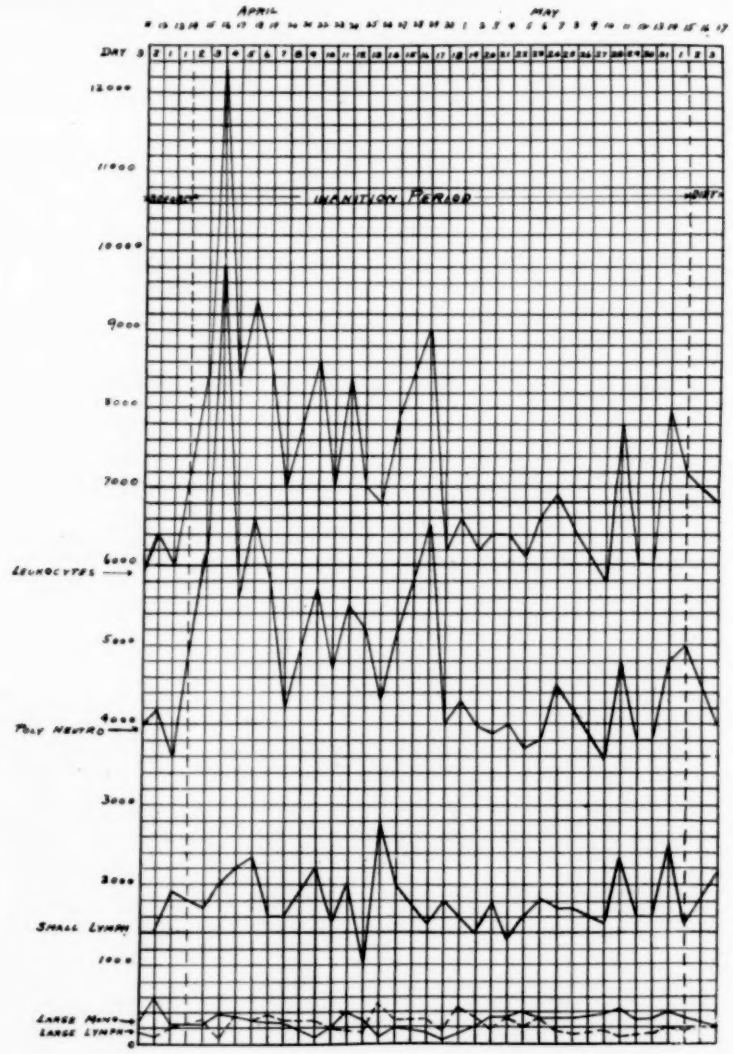

Fig. 3.—Relation of total white count to differential.

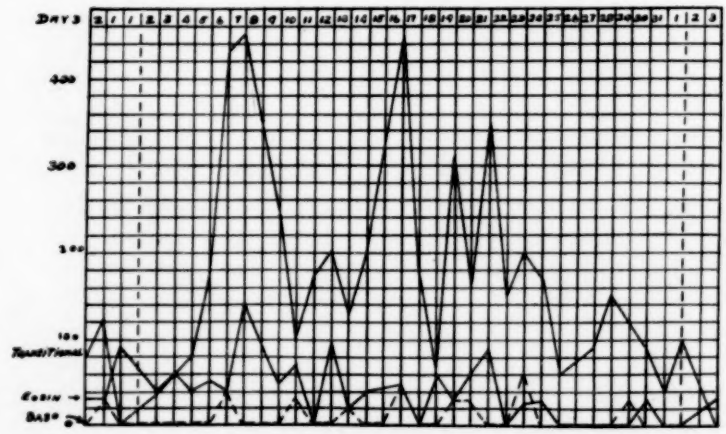

Fig. 4.—Transitionals, eosinophils and basophils.

Leukocytes.—It is in the total leukocyte count that we have the most striking change of the whole series of examinations. There was a rapid rise at the onset of the fast, reaching 12,400 on the third day. On the fourth day, however, it immediately fell to 8,400, after which there was a consistent daily variation of about 1,000 until the sixteenth day, when it reached approximately the preliminary count, after which there will be noticed a more marked daily variation. It was possible to continue the examinations for only three days following the fasting period so that the count had not settled down to normal when the subject went out from under observation.

Polymorphonuclear Neutrophil.—These ran throughout quite consistently with the total count, and the marked variations in this latter were quite apparently due to the change in polynuclear content.

Small Lymphocyte.—There is a number of fluctuations in the count of this cell, except during the period between the fourteenth and twenty-seventh days, when they were quite constant, as were also the total and polymorphonuclear counts. It will be noted that the majority of the rises and falls are the opposite of those seen in total and polymorphonuclear curves. This is particularly the case on the second, fourth, thirteenth, fourteenth, sixteenth, seventeenth, twentieth and twenty-first days of fast and the first day of refeeding. It seems safe to conclude, therefore, that these fluctuations are only relative, being due really to the fluctuations in polymorphonuclears and that their number was practically constant throughout the fast. The other forms present no distinctive features except the transitional, which was subject to several rises, namely, on the seventh, sixteenth, nineteenth and twenty-first days, when they were about 5 per cent. Only an occasional eosinophil was found during the last ten days of the fast and they had not returned to their usual number when examinations were discontinued. By examination of Figure 2 there appears to be a very slight increase in the combined mononuclear cells throughout the fasting period, the average being raised by the fluctuations in the transitional form, for the other types, that is, monocytes, large and small lymphocytes, are practically constant throughout except the variation already noted in the latter.

Coagulation-Time.—Toward the end of the second week of inanition, it was noticed that the blood coagulated more rapidly than it had during the earlier days. It became more noticeable each day so that if the mixing pipets were not filled very rapidly, the drop would coagulate, or the blood would clot in the tubes. It is certain that this was not due to any physical alteration of the patient's environment. The temperature of the balcony where the subject stayed and where

the estimations were made was practically constant. This is a very important factor, for most experimental evidence goes to show that variations in temperature have decided influences on the coagulation time.[69, 70]. On the seventeenth day the estimations of the time were begun. Until the twenty-fifth day the McGowan method was used. Capillary tubes of uniform caliber were filled with blood escaping after the specimens for the other examinations had been obtained, practically always the same relative drop, the third. Small sections were broken off at intervals of from ten to thirty seconds, and when a fine filament of fibrin was observed between the carefully separated ends of the tube and fragment, the time elapsed since the appearance of the drop used was taken as the reading. For the remainder of the examinations, the Boggs[71] apparatus was used.

TABLE 3.—Protocol of Coagulation Time of Blood

No. Day	Coagulation Time		Remarks
	Levanzin	Control	
17	1′ 5″	3″	McGowan method.
18	1′20″	2′50″	McGowan method.
19	1′20″	2′	McGowan method
20	1′ 5″	.....	McGowan method.
21	55″	1′55″	McGowan method.
22	1′20″	.....	McGowan method.
23	1′ 5″	1′35″	McGowan method.
24	1′ 5″	4′20″	Boggs used on control.
25	.....	4′50″	From this day on Boggs
27	3′20″	4′+	used on both.
28	2′50″	.....	
29	2′20″	.....	
30	1′50″	3′30″	
31	2′30″	.....	
First diet	2′45″	6′20″	

The figures as obtained by these two methods, together with those found in a normal individual examined on the same days, with the same methods and under approximately the same conditions, are collected in Table 3. The last record on the control was made in a temperature at most 2 degrees C. cooler than that of the subject's environment, but a deduction of two minutes as correction for this still leaves the time distinctly longer than that obtained on the same day in Levanzin's blood. A comparison of the two series of results

69. Hartman, J.: Zur Frage der Blutgerinnenzeit, München. med. Wchnschr., 1909, lvi, 796.
70. Rudolf, R. D.: Tr. Assn. Am. Phys., Philadelphia, 1910, xxv, 504.
71. Boggs, T. R.: Johns Hopkins Hosp. Bull., 1904, xv, 174.

will demonstrate a distinct increase in the coagulability of the starving man's blood, more noticeable toward the end of his fast.

Specific Gravity.—The specific gravity was determined on only two occasions, the first while the subject was eating his first meal after fasting and the second on the third day of refeeding. On the former occasion it was 1.0612 and on the latter, 1.0618. The estimations were made by the Hammerschlag[72] method, the specific gravity of the mixture of chloroform and benzol being determined by the pyknometer. As no figures were obtained either before or during the fast, these two examinations are of little value except that from them it may be assumed that there is a very slight increase in density, taking 1.059 to 1.060 as the average normal specific gravity.

DISCUSSION AND CONCLUSIONS

The results of the preceding studies are conspicuous rather from the absence than from the presence of striking alterations in the blood-picture. Really the only prominent features are the early rise in polymorphonuclear neutrophils and the decrease in coagulation time. The leukocytes, that is, the neutrophils at least, are the most sensitive of the blood-cells to changes in body conditions and we know that apparently slight disturbances—a cold bath, for example—will call forth a recognizable increase in these cells. They seem always to be on the alert, ready at the least evidence of disturbance to rush forth in defense of the organism. It is scarcely to be wondered at, therefore, that in response to such an unusual condition as starvation there should be an outpouring of the reserve supply, at least for a day or so, or until the organism has had an opportunity to adapt itself to the altered conditions. The variations in water content of the blood cannot be considered a factor in this rise, involving as this does only the one form of cell. The only explanation that suggests itself therefore, and frankly not a particularly scientific one, is this alertness of the polymorphonuclear and its constant readiness to be on the defense for the organism. The products of the somewhat perverted metabolism may excite them into this early activity and later fail to do so, but there is no evidence to prove this supposition. It is not easy to understand why they should respond to toxic products in the early days and not the later as well, unless we assume they acquire a tolerance for them, which seems improbable when we compare the reaction to infections, in which their fight is evident throughout the disease if the organism is to conquer.

As to the effect of the starving on the total quantity of blood, it does seem evident that there are fluctuations at least in the first

72. Hammerschlag: Wien. klin. Wchnschr., 1890, iii, 1018.

two weeks. It will be noted by comparing the curves of the white and red cell-counts the variations are synchronous, that on the third, fifth, ninth and eleventh days particularly, the noticeable increase in the one are accompanied by equally frank rises in the other. It would seem that this could be due only to variation in water content. The specific gravity would have gone far to prove this point, but unfortunately this was not determined during this period. Taking these fluctuations as indication of variation in water content, it appears that during the middle period of the fast, at least, the equilibrium of intake (including that drawn from tissues) and output was pretty well established.

The hemoglobin appears to be particularly resistant, the percentage on the last day being within 2 per cent. of the highest estimation, found on the day before fast began, though there was a moderate decrease during the second ten days.

It is difficult to account for the only other marked change in the blood, that of increase in coagulability. Loss of water could be responsible, but there is no evidence that this occurred. The very slight increase in density at the last, determined by the specific gravity, certainly does not demonstrate a sufficient concentration. There were no estimations of the platelet content made, but it is quite possible that the explanation lies with these. An increase in them could be responsible.

The final conclusions as to the effects of starvation on the blood to be drawn from the results of examinations on Levanzin are:

1. There is a slight actual loss in hemoglobin, more marked during second ten days.

2. There are moderate fluctuations in water content, particularly during first half of period.

3. There is a decided rise in polymorphoneutrophils in the early days.

4. There is an increase in coagulability, especially after the first two weeks.

5. The blood as a whole is distinctly resistant to the effects of uncomplicated inanition, permitting of entire abstinence from food for long periods of time (at least thirty-one days) without deleterious consequences, as far as the blood is concerned.

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THE PATHOLOGIC PHYSIOLOGY OF THE CIRCULATION DURING HEMORRHAGE *

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NEW YORK

A number of authors, Marey,[1] Fredericq,[2] Lazarus,[3] Crile[4] and Henderson[5] among them, have presented either a review or their conceptions of the changes produced in the circulation during hemorrhage. Each of these writers has reflected the knowledge available at the time and much that has already been said can merely be reechoed. For a number of years, however, I have been collecting experimental evidence which, in some instances, extends or interrelates the observations already established and, in others, appears to necessitate a revision of current views. Hence, although many problems still await investigation, the attempt is made to present such a picture of the pathological changes in the circulation during hemorrhage as appears justifiable in the light of present-day knowledge. In doing this the hope is entertained that such an exposition may prove of value in interpreting the signs or symptoms that present themselves at the bed-side and in a certain measure also govern the treatment.

I. THE SEQUENCE OF EVENTS DURING HEMORRHAGE

When an artery of considerable size ruptures, the total resistance against which blood is discharged is reduced. Consequently, a fall in diastolic pressure results. The systolic pressure also decreases though not to the same extent as the diastolic. Hence, the pulse-pressure increases (Fig. 1). There are several reasons for this; in the first place, the "effective pressure" in the left auricle,[6] which

* From the Physiological Laboratories of the University of Michigan, Ann Arbor, and Cornell University Medical College, New York City.
* Submitted for publication, March 23, 1914.

1. Marey: Circulation du Sang, 1882, p. 192.
2. Fredericq: Travaux du Laboratoire de Liège, 1885-86.
3. Lazarus: Diseases of Blood, Nothnagel's Practice, Am. Ed., Philadelphia, 1909, p. 154.
4. Crile: Hemorrhage and Transfusion, 1909, New York, pp. 63 and 96.
5. Henderson: Am. Jour. Physiol., 1910, xxvii, 155.
6. The term "effective pressure" has been applied by Henderson and Barringer (Am. Jour. Physiol., 1913, xxxi, p. 288) to the difference between intra-auricular and intrathoracic pressures and it is this pressure and not the actual auricular pressure which determines the filling of the ventricle.

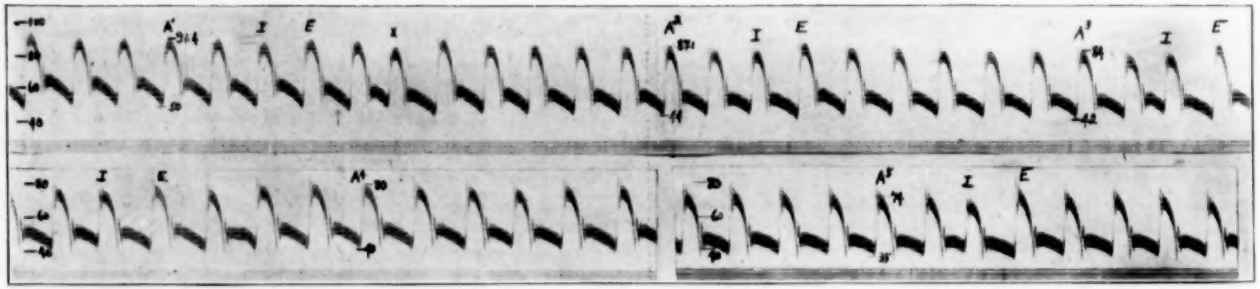


Fig. 1.—Effect of hemorrhage on pulse-pressure taken from carotid by author's optically registering manometer (—— actual size). *I*. Records showing effect of moderate hemorrhage begun at arrow (upper record). Observe that systolic and diastolic pressures vary with inspiration *I* and expiration *E* as compared with the period of intervening quiet. Comparing corresponding waves (A^1, A^2, A^3) in upper curve it is found that systolic and diastolic pressures fall, but pulse-pressure is increased. Later in hemorrhage (lower curves) when both systolic and diastolic pressures have fallen more it is found that the pulse-pressure is decreased.

largely determines the filling of the left ventricle, is not immediately reduced by a diminished supply of blood, hence, the ventricular output is not diminished. In fact, the observations of Johanssen and Tigerstedt[7] indicate that the systolic discharge increases. In the second place, the volume of elasticity coefficient of the arteries becomes greater at lower pressures, hence, the same systolic discharge will give a greater pressure variation. The amplitude of the pulse, which is determined by the pulse-pressure, accordingly becomes large and the shape of the peripheral pulse, which is determined by the low resistance, assumes the collapsing form.

While the left heart is thus maintaining fully a normal discharge and the pulse-pressure is greater, the peripheral flow of blood into the veins is almost immediately reduced. At first, the right auricular pressure is not affected, probably because the reduced volume of

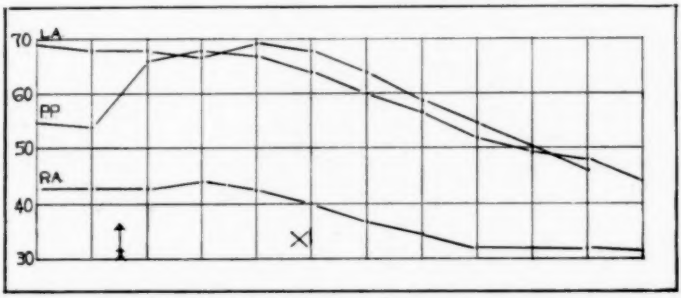


Fig. 2.—Plot from Experiment 377, showing immediate effect of a small hemorrhage at arrow on pulse-pressure (*P.P.*), on effective left auricular pressure (*L.A.*), and effective right auricular presure (*R.A.*) Figures indicate millimeters of mercury when applied to curve of pulse-pressure and millimeters of saline when applied to auricular pressures. Abscissae equal ten second intervals.

venous blood from one region is compensated by an increased flow from another in much the same manner as in clamping the inferior vena cava more blood flows through the external jugular. When the luxus blood-content of the veins has been somewhat reduced, however, this balance of venous flow is lost and the "effective right auricular pressure" is decreased (Fig. 2). Almost simultaneously the volume curve of the entire heart diminishes in amplitude,[8] pulmonary arterial and left auricular pressures fall and the systolic pressure becomes rapidly smaller until the pulse-pressure is less than normal.

The flow through the organs of the body is slowed and the centers in the medulla are stimulated by the reduced blood-flow. Investi-

7. Johannsen and Tigerstedt: Skandin. Arch. f. Physiol., 1889, i, 394.

8. Wiggers, C. J.: The Prognostic Significance of Pulse-Presure Changes During Hemorrhage, Arch. Int. Med., 1910, vi, 281. (See Fig. 1.)

TABLE 1.—Experiment C 18*

Type of Breathing	Auricular Pressure				Intrathoracic Pressure		Effective Pressure (Auricular)			
	Right		Left				Right		Left	
	Insp.	Exp.	Insp.	Exp.	Insp.	Exp.	Insp.	Exp.	Insp.	Exp.
Natural, quiet	55	62	—20	0	— 62	—30	117	92	42	30
Tracheal stimulation (deeper breathing)	50	65	—46	0	— 94	—28	144	93	48	28
Natural breathing (12 min. after)....	60	66	—16	+ 8	— 48	—24	108	90	32	32
Sciatic stimulation (85 seconds).....	66	80	—32	+18	—130	+ 2	196	78	98	16
Apnea (1 min. duration)	66	..	0	....	— 33	....	99	..	33	

* Experiments arranged according to average loss per minute.

gators[9] are not entirely agreed whether an altered blood-flow may affect the bulbar centers without a change in the general gaseous content of the blood, but the increased respiratory movements following the use of pressure-lowering agents has convinced me that such a direct effect exists.

While these dynamic changes are taking place, a series of important chemical alterations occur in the blood. The experiments of Mathieu and Urbain,[10] Bert,[10] Vinay[10] and Finkler[11] all indicate that the loss of red cells from the circulation and the consequent reduction of the oxygen-carrying power, aided by the slowed circulation in the peripheral capillaries lead, first, to a decrease in the oxygen content of the venous and later of the arterial blood, while in neither is the

TABLE 2.—Relation of Cardiac Rate to Rate of Loss of Blood

Exp.* No.	Weight kg.	Duration min.	Total Loss c.c.	Percentage Loss	Avge. Loss per min. c.c.	Heart Rate		Pct. Gain
						Before	After	
153	10.0	35	150	1.5	4.3	135	105	
151	10.4	36	208	2.0	5.7	148	112	
176	8.5	37	212	2.5	5.7	99	99	
160	10.1	33	148	1.5	6.4	105	102	
163	6.1	6	60	1.0	10.0	111	135	21.8
178	10.5	35	352	3.5	10.0	108	135	25.0
365	17.0	30	570	3.0	19.0	150	220	46.6
350	9.0	3	90	1.0	30.0	78	102	30.8
359	10.8	5	270	2.5	54.0	105	156	48.6
341	10.0	4	350	3.5	87.0	150	182	21.3
344	17.0	1	170	1.0	170.0	138	156	13.0
364	15.0	1.5	300	2.0	200.0	96	162	75.0

* All figures in millimeters of water.

percentage of carbon dioxid materially altered. Some recent gas analyses, performed in this laboratory by Murlin and reported in part by Murlin and Greer,[12] confirm these observations with more recent methods of analysis.

This reduction in the oxygen percentage of arterial blood operates with the reduced flow to increase the activity of the respiratory center. At first the respirations increase in rate and depth, giving rise for a considerable interval to hyperpnea which is probably attributable to the decreased flow, with perhaps a local concentration of the carbon

9. Guthrie and Pike: Am. Jour. of Physiol., 1906, xvi, 480; 1908, xx, 451; Eyster, Austrian and Kingsley: Am. Jour. Physiol., 1907, xviii, 413.
10. Quoted by Fredericq: Travaux du Laboratoire de Liège, 1885-86.
11. Finkler: Arch. f. d. ges. Physiol., 1875, x, 369.
12. Murlin and Greer: Am. Jour. Physiol., 1914, xxxiii, 253.

dioxid in the medulla.[13] The greater depth and rate of respirations thus instituted cause, to judge from the variations in pulse-pressure, a greater average discharge of the left ventricle.[14] No explanation of the mechanism involved is as yet entirely satisfactory. I[14] have previously published records[15] which indicated that an increase in rate and depth of respiration caused no alteration in the right auricular pressure during expiration, but that the more negative intrathoracic pressure during inspiration caused the "effective pressure" to increase. This has been shown by recent experiments to be true of both right and left auricular pressures. Probably no appreciable increase in the blood-supply of the two auricles takes place during expiration until

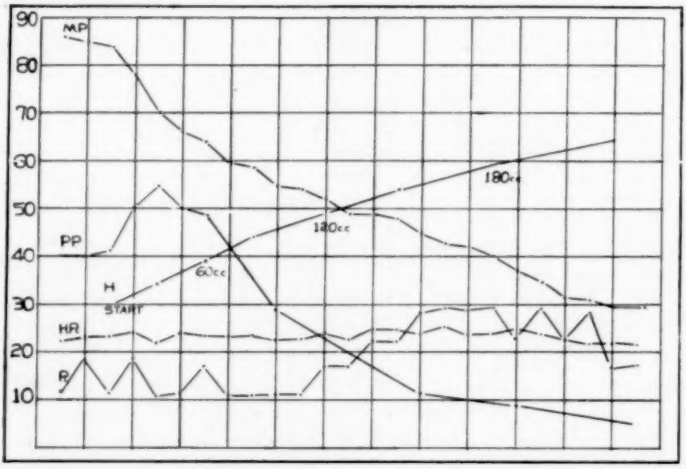


Fig. 3.—Plot from experiment 359, showing effect of a hemorrhage (indicated by line *H*) on mean arterial presure (*M.P.*), pulse-pressure (*P.P.*), heart-rate (*H.R.*) and respiration-frequency (*R*), the latter two estimated for ten second intervals. Abscissae equal ten second intervals.

forced abdominal movements occur; but the more frequent occurrence of the more negative inspiratory phase with its greater "effective pressures" favors the *average venous filling* pressure and increases the *average ventricular ejection.*

This tendency of increased respiration to increase the "effective venous" pressure was at first thought to account for the influence of augmented breathing on arterial pressure. That some other mechanism is further concerned, however, is to be assumed, since the arterial

13. Paul Bert: La pression barométrique, Paris, 1878, p. 1061.
14. Wiggers, Eberley and Wenner: Jour. Exper. Med., 1912, xv, 67.
15. For example, Plate 27, Figure 3, Jour. Exper. Med., 1912, xv, 67.

pulse-pressure is smallest in inspiration, that is, when the effective pressures in both auricles are highest.

The altered circulation through the medulla affects also the cardio-inhibitory and vasomotor centers (Plot, Fig. 3). The acceleration of the heart during hemorrhage has been repeatedly observed since Hales,[16] Pawlow, von Leser and others directed attention to this phenomenon and its importance in the maintenance of blood-pressure is generally recognized. The additional work of Fredericq,[17] Hayem[18] and others, has shown that the acceleration results from a depression of the cardio-inhibitory center. Dawson[19] has pointed out, however, that the acceleration is not equally pronounced in all cases, depending on the initial tonus of the cardio-inhibitory center. I have been able to confirm this and, in addition, it appears from the tabulated results that, in a certain measure, the cardiac acceleration is related to the rate at which blood is lost. The data contained in Table 2 indicate that, while the percentage increase in cardiac rate is not related to the actual quantity of blood lost, or to the amount lost in relation to body weight, it is related to the average rate of loss.

Stimulation of the vasomotor center causes an increased contraction of the peripheral blood-vessels during hemorrhage (Sollmann and Pilcher[20]). Such a tendency to increase the total resistance by constriction is antagonized by the reduction in the viscosity of the blood. Cope[21] has shown that, in spite of this, the total resistance remains much increased. Little more than inference seems to support the commonly held idea that the arterial walls contract through vasomotor influence and thus adapt their caliber to the reduced volume of blood.[22] The demonstration of such an arteriomotor reaction by faultless methods would be of great importance. The accommodative diminution in the larger arteries is more probably due to an elastic recoil of the vessels, which is even more favorable, for while the vessels draw together when the pressure lowers, no increased resistance to the flow results, such as would be induced by a general vasoconstriction.

To sum up, the fall of arterial pressures is counteracted by the augmented breathing, an accelerated heart, an accommodative con-

16. Hale: Hemostatiks, 1733, ii, 2.
17. Fredericq: Travaux du Laboratoire de Liége, 1886; Arch. du. Biol., 1882, iii, 55.
18. Hayem: Arch. de physiol norm. et path., 1886, i, 106.
19. Dawson: Jour. Exper. Med., 1905, vii, 6.
20. Sollmann, T., and Pilcher, J.: Am. Jour. Physiol., 1910, xxvi, 233.
21. Cope: Am. Jour. Physiol., 1911, xxix, 137.
22. See for example, Starling: The Principles of Human Physiology, 1912, p. 1131. Phil. and New York.

traction of the larger vessels and by a peripheral constriction leading to an increased resistance in spite of the reduction in viscosity. All the factors enumerated tend not only to keep up the arterial pressure but also to increase the flow through the tissues with the exception of the increased resistance, which tends to diminish the peripheral flow. So great, however, is this single factor that the volume of organs and the venous outflow are always greatly reduced.

If this is so, it may be asked, to what purpose does such a compensatory mechanism exist? Evidently all the pressure-maintaining mechanisms are brought into play so that the brain, with its more feebly innervated vessels, (Wiggers[23]) shall remain perfused by a supply as nearly normal as possible. The experiments of Gaertner and Wagner,[24] Roy and Sherington,[25] Bayliss and Hill[26] and others have shown adequately the direct dependence of the cerebral blood-flow on the general arterial and venous pressures. As arterial pressure is reduced and the cerebral flow decreases, the compensatory reactions not only elevate the general arterial pressure but also divert the blood from the less sensitive tissues to the brain with its vital centers.

It is of further interest to note that the increased rate of cardiac contraction and perhaps the decreased intraventricular tension in themselves supply mechanisms for insuring an adequate blood-flow through the coronaries so that a deficient nourishment and impaired reserve power such as Crile[27] describes does not exist until late in hemorrhage. Many times by the method utilized to establish the innervation of the coronary vessels in the intact circulation, I have observed[28] that the coronary flow after hemorrhage equaled or even exceeded the normal flow, even though the arterial pressure was less than normal (Fig. 4). In this way, it may be explained why the heart is able to maintain a normal contractile power as shown in cardiographic tracings after considerable loss of blood has taken place.

II. THE MECHANISM OF HEMORRHAGE CESSATION

Every hemorrhage, except one coming from a very large vessel, has a natural tendency to be checked long before the loss of blood becomes dangerous. The duration of bleeding depends not so much on the size of the vessels wounded as on the facility with which an efficient clot forms over the opening. When blood is shed, it begins

23. Wiggers: Am. Jour. Physiol., 1908, xxi, 454.
24. Gaertner and Wagner: Wien. med. Wchnschr., 1887, pp. 602, 639.
25. Roy and Sherington: Jour. Physiol., 1890, xi, 85.
26. Bayliss and Hill: Jour. Physiol., 1895, xviii, 356.
27. Crile: Hemorrhage and Transfusion, New York, 1909, p. 96.
28. Wiggers: Am. Jour. Physiol., 1909, xxiv, 400.

to coagulate on the tissues at a distance from the opening. Having formed a point of adhesion to the tissues, it is added to by accretion until it reaches and covers the wound. The factors which determine the distance of the first clot formation and its development are, therefore directly concerned with the duration of hemorrhage.

Other factors being equal, the pressure at the arterial opening determines the distance that blood will be ejected before the force is sufficiently spent to permit the coagulum to form. A very pretty experiment may be performed by allowing a tiny stream of arterial blood to play on a glass slide and impinge against a small elevation cemented to it. The process of clot development may then be watched under a low-power objective. The struggle between the adhesion of agglutinated red cells to the formed clot and the force of the pressure of the stream which attempts to detach them comes clearly before one's eyes. The development of intertwining fibrin threads, making

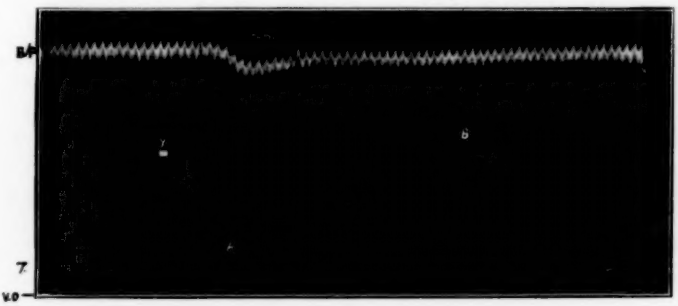


Fig. 4.—Curves from experiment 254, showing effect of a sudden femoral hemorrhage at *X*. The flow through the coronaries indicated by the slope of the curve *V.O.* towards the abscissae at first decreases at *A* but later increases at *B*, in spite of the fact that the arterial pressure (*B.P.*) is less than before bleeding.

these adhesive masses finally secure and a part of the clot, is of extreme interest. If the pressure of the spurting stream is suddenly increased, unfastened masses may be torn loose and new cells adhere much more slowly. After this fashion the blood-pressure influences the cessation of hemorrhage in the body. Attention may be directed in this connection to the fact not generally emphasized that a clot once formed is called on to resist not the mean but the maximal pressure that exists in the artery. Now, since in conjunction with Eberly and Wenner[29, 30] I have found that the maximal pressure in the systemic as well as in the pulmonary circuit is often entirely determined

29. Wiggers, Eberly and Wenner: Jour. Exper. Med., 1912, xv, 174.
30. Wiggers: Am. Jour. Physiol., 1912, xxx, 233.

by the depth of respiration, it is quite possible that a drug which, in a normally breathing animal, increases the cardiac output and causes a rise in the average mean pressure may, nevertheless, by reducing the dyspnea during hemorrhage actually lessen the maximal pressure in the artery.

The surface on which blood is shed determines the extent of its spread. Thus, the smooth surface on which the intestinal and uterine vessels rupture compares unfavorably with the reticular lung parenchyma and subcutaneous connective tissue. So, also, the spread may be limited by an ulceration or excavation.

The coagulation time directly influences the course of clot-formation. Our conception of the chemical processes has undergone radical modification since the recent work of Howell[31] on this subject. According to this investigator we find, in the circulating fluid, fibrinogen, prothrombin, calcium salts and antithrombin. The last-mentioned substance holds the prothrombin in combination and this prevents its conversion to thrombin. When the blood is shed the disintegrated corpuscles, blood-platelets and the tissues with which blood comes into contact, furnish thromboplastin (chemically a phosphatid resembling kephalin in its properties) which, by combining with antithrombin, liberates prothrombin. This can now be activated by calcium and acts on fibrinogen. Any excess formation of antithrombin (Whipple[32]) or lack of prothrombin such as occurs in hemophilia (Howell[33]) may delay clot formation and interfere with the cessation of hemorrhage.

The formation of clots may be retarded or prevented by muscular contractions of organs. The regular cardiac contraction, as a rule, absolutely prevents any clot formation over wounds of the heart and forms the only serious element in such wounds. The rhythmic peristalsis in the stomach and intestines are very unfavorable for clot formation and the induction of these movements in the intact abdomen by injections of epinephrin may possibly be sufficient to counterbalance any such favorable vascular influence as were indicated by my previous laboratory experiments.[34] On the other hand, the only hemostatic value of atropin resides in its power to inhibit muscular movements and its use *in conjunction with vascular agents* such as epinephrin in hemorrhage from muscular organs is physiologically indi-

31. Howell: Am. Jour. Physiol., 1908, xxi, 51; 1912, xxxi, 1.
32. Whipple, G. H.: Hemorrhagic Disease: Antithrombin and Prothrombin Factors, ARCH. INT. MED., 1913, xii, 637.
33. Howell, W. H.: The Conditions of the Blood in Hemophilia, Thrombosis and Purpura, ARCH. INT. MED., 1914, xiii, 76.
34. Wiggers, C. J.: Adrenalin in Intestinal Hemorrhages, ARCH. INT. MED., 1909, iii, 177.

cated, especially, as the increasing percentage of carbon dioxid in the blood itself tends to increase the rhythmic contraction of organs.

III. EVENTS FOLLOWING CESSATION OF HEMORRHAGE

When hemorrhage terminates favorably by coagulation, the volume of blood returned to the right auricle increases with surprising rapidity. This is shown by the ventricular volume curves[35] as well as by the increase in venous pressure quickly following the cessation of hemorrhage (Fig. 5). The immediate cause of this increase is probably found in the prompt rise of arterial pressure following clot formation or application of a hemostat (R^1, Fig. 6) together with the rapid reabsorption of lymph to replete the blood-volume. Incidentally it may be pointed out that this dilution of the blood decreases the number of red cells and diminishes the percentage of hemoglobin (Dawson), thus explaining the posthemorrhagic decrease in both for several days. Practically nothing is yet proved as to the existence of an adaptive contraction of the larger veins in favoring the return of blood, yet the existence of such a posthemorrhagic contraction can be conceived of in view of Mall's[36] interesting contributions to the innervation of large veins.

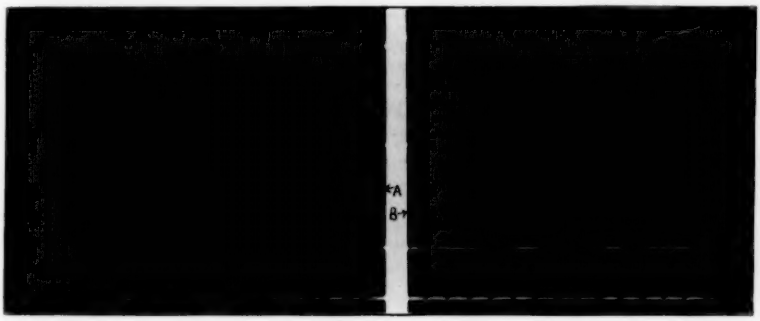


Fig. 5.—Curve showing right (lower) and left, auricular pressures (upper) during hemorrhage (A) and 48 seconds after at B. One mm. record = 4.3 mm. saline.

In consequence of the better venous supply and greater output of both ventricles, the pulse-pressure increases, for there is little alteration in the peripheral resistance and the increase in the volume elasticity coefficient remains an almost negligible factor. Both systolic and diastolic pressures thus gradually rise, owing to the augmented

35. Wiggers, C. J.: The Prognostic Significance of Pulse-Pressure Changes During Hemorrhage, Arch. Int. Med., 1910, vi, 281.
36. Mall: Arch. f. Physiol., 1892, p. 409.

cardiac output—a change that is mirrored as the secondary rise of mean pressure at R^2 (Fig. 6). The assumption is generally made that this posthemorrhagic increase in mean pressure is further assisted by an additional vasoconstriction; indeed, it is often quoted as the chief or only cause for the pressure recovery. All experimental observations made by the method described by Cope[21] have indicated to me *that vasoconstriction is a gradual process, starting while hemorrhage is still in progress and continuing after its cessation.* Neither experiments nor *a priori* reasoning, however, warrant the assumption that a greater vasomotor reaction after hemorrhage is at all likely to occur, nor is such an assumption necessary to explain this secondary rise.

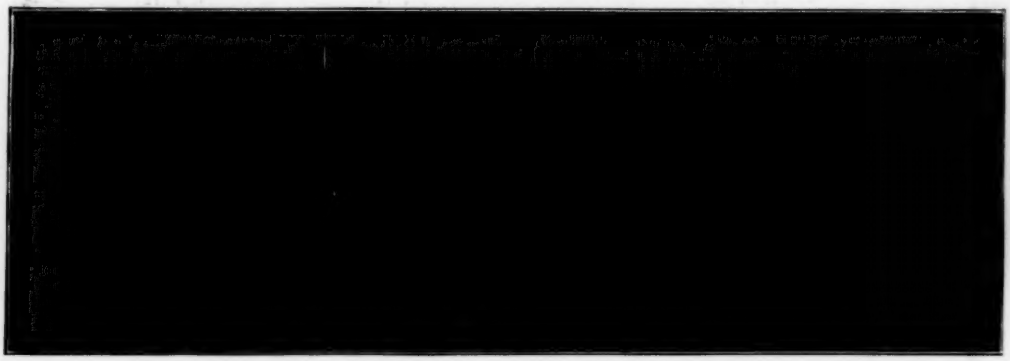


Fig. 6.—Curve from experiment 334, showing effect of femoral hemorrhages on carotid pressure (*P.A.*) pulmonary arterial pressure (*P.A.*) and on cardiometer tracing *V*. *X*-*X'* small hemorrhage. *X'* to *R'* large hemorrhage. Clamp applied to artery at *R'*. Posthemorrhagic rise at R^2.

The rise of arterial pressure, together with the continued dilution of the blood, tends to increase the flow both in the peripheral tissues and in the medulla. The overstimulation of the respiratory center gradually ceases and the respirations become normal again (Fig. 6). The heart continues rapid, however, and the peripheral vessels remain contracted until sufficient regeneration of the red blood-cells has taken place to reestablish normal conditions.

IV. EVENTS IN THE TERMINAL STAGES

If coagulation fails to occur, the time soon comes when the reduction in blood-volume exceeds the possibilities of lymph absorption, to such an extent that the mechanical function of the circulation cannot be efficiently carried out. The amplitude of cardiac contraction then decreases, largely because fluid is lacking to feed the pump. As has been recently pointed out elsewhere,[37] the reduced "initial tension"

which this occasions is in itself sufficient to decrease the rate and vigor of the systolic contraction. The reasons for saline infusion or blood transfusion is obvious. Epinephrin also favors the rapidity of cardiac contraction in such cases and so improves the arterial pressure.[37]

The lack of oxygen in the blood soon changes the previous stimulation of the respiratory center to a depression (Fig. 7). Slow and shallow breathing, occasionally marked by a deeper gasp, leads to complete cessation. The "pressor influence" of augmented breathing is removed and the blood-pressure falls rapidly. Asphyxial changes

I II III IV V VI VII VIII IX

Fig. 7.—Nine segments showing effects on respiration (upper curve) and blood-pressure (lower curve) of a progressive hemorrhage. I. Normal breathing and blood-pressure. II. Deeper breathing with greater respiratory variation in blood-pressure. III. Accelerated deeper breathing. IV. Irregular breathing following temporary decrease in hemorrhage. V. Decreased breathing due to depression of respiratory center. VI. Slow, shallow breathing. VII. Apnea. VIII. Asphyxial contractions of heart. IX. Asphyxial movements of respiration. Heart very slow and irregular.

in the circulation terminate the case. The heart-beats are slow but of great amplitude and vigor, thus making a last attempt to elevate the pressure, which is partly successful but soon fails for want of oxygen.

37. Wiggers: Am. Jour Physiol., 1914, xxviii, 382. (See Figures 4 and 7.

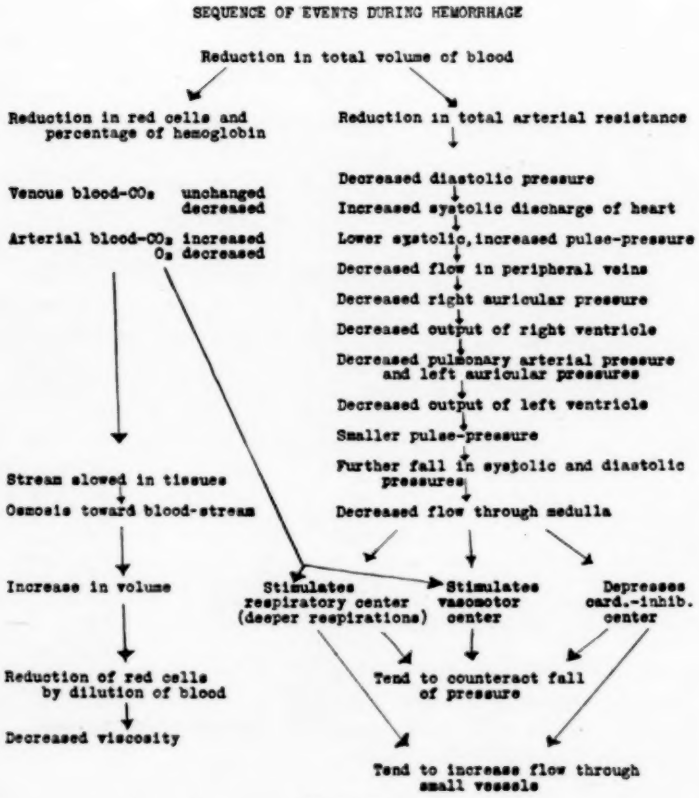
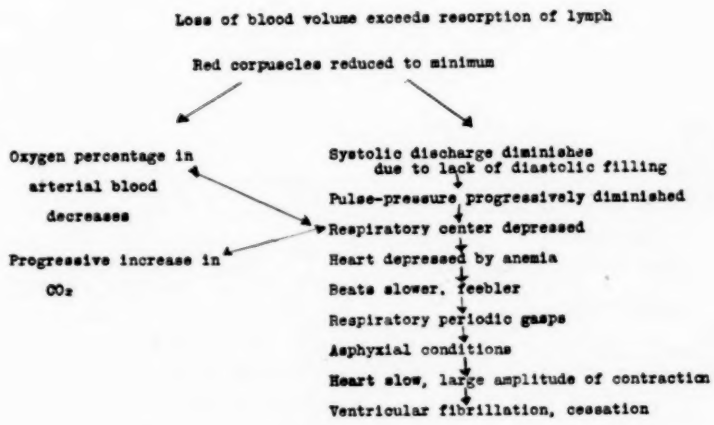

SEQUENCE OF EVENTS DURING HEMORRHAGE
Reduction in total volume of blood
Reduction in red cells and percentage of hemoglobin
Reduction in total arterial resistance
Venous blood-CO2 unchanged decreased
Arterial blood-CO2 increased O2 decreased
Decreased diastolic pressure
Increased systolic discharge of heart
Lower systolic, increased pulse-pressure
Decreased flow in peripheral veins
Decreased right auricular pressure
Decreased output of right ventricle
Decreased pulmonary arterial pressure and left auricular pressures
Decreased output of left ventricle
Smaller pulse-pressure
Further fall in systolic and diastolic pressures
Stream slowed in tissues
Osmosis toward blood-stream
Decreased flow through medulla
Increase in volume
Stimulates respiratory center (deeper respirations)
Stimulates vasomotor center
Depresses card.-inhib. center
Reduction of red cells by dilution of blood
Tend to counteract fall of pressure
Decreased viscosity
Tend to increase flow through small vessels

Figure 8.

SEQUENCE OF EVENTS DURING TERMINAL STAGES


Loss of blood volume exceeds resorption of lymph
Red corpuscles reduced to minimum
Oxygen percentage in arterial blood decreases
Progressive increase in CO_2
Systolic discharge diminishes due to lack of diastolic filling
Pulse-pressure progressively diminished
Respiratory center depressed
Heart depressed by anemia
Beats slower, feebler
Respiratory periodic gasps
Asphyxial conditions
Heart slow, large amplitude of contraction
Ventricular fibrillation, cessation

SEQUENCE OF EVENTS IN CESSATION OF HEMORRHAGE

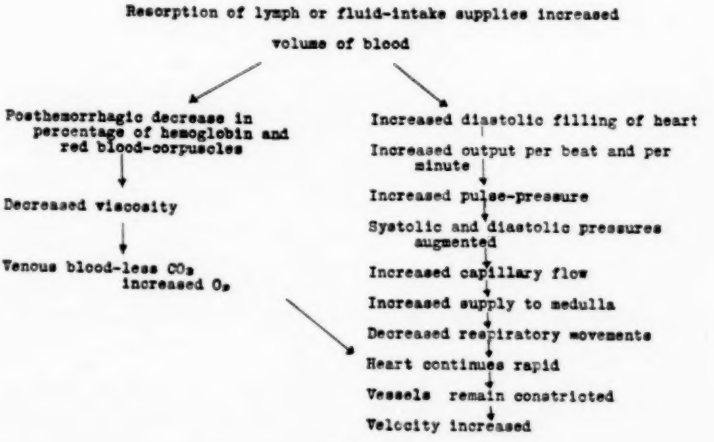

Resorption of lymph or fluid-intake supplies increased volume of blood
Posthemorrhagic decrease in percentage of hemoglobin and red blood-corpuscles
Decreased viscosity
Venous blood-less CO_2 increased O_2
Increased diastolic filling of heart
Increased output per beat and per minute
Increased pulse-pressure
Systolic and diastolic pressures augmented
Increased capillary flow
Increased supply to medulla
Decreased respiratory movements
Heart continues rapid
Vessels remain constricted
Velocity increased

Figure 9.

THE BLOOD-PRESSURE IN PNEUMONIA*

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For decades clinicians have believed that many of the grave symptoms and a large number of the deaths in pneumonia were directly due to failure of the circulation. In 1889 Romberg[1] and his associates studied the problem and reached the conclusion that, while fatal circulatory failure did occur in rabbits inoculated with lethal doses of pneumococci, the failure was not of myocardial origin as had been supposed, but was invariably caused by the exhaustion of the vasomotor center in the bulb.

Since that time physicians have tried to combat the failure of the vasomotor mechanism in pneumonia by administering drugs which would augment the activity of the vasomotor center.

The results have been sufficiently confusing to make a re-investigation desirable. The present communication deals only with the blood-pressure in pneumonia. Other phases of the subject will appear in later papers.

If it be true that grave symptoms and death in pneumonia be caused by failure of the vasomotor apparatus, then it ought to be possible to divide cases of lobar pneumonia into two groups:

1. Those in which the vasomotor apparatus is intact.
2. Those in which the vasomotor apparatus is seriously injured.

In the first group the blood-pressure measurements ought to fall on a curve which does not diverge widely from that for normal individuals. In the second group the blood-pressure curve ought to be strikingly below the normal and to fall still lower as the gravity of the vasomotor disturbance increases. The blood-pressure curve ought to be the most reliable single measure of the state of the circulation in pneumonia, and a definite and exact indicator for our therapeutic efforts.

In 1911 Weigert[2] studied the blood-pressure in a variety of infectious diseases, including pneumonia, and reviewed the work of the

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* From the East Medical Service of the Massachusetts General Hospital.
† Aided by grant from the Council on Pharmacy and Therapeutics of the American Medical Association.

1. Romberg, Pässler, Bruhns and Müller: Experimentelle Untersuchungen über die allgemeine Pathologie der Kreislaufstörung bei acuten Infectionskrankheiten, Arch. f. klin. Med., 1899, lxiv, 652.

2. Weigert, K.: Ueber das Verhalten des Arteriellen Blutdrucks bei den acuten Infectionskrankheiten, Samml. klin. Vortr. (Volkmann's) Inn. Med., No. 138, p. 459.

earlier observers. He found that the statements of his predecessors were so contradictory that he could draw no conclusions from them. Among his own thirty-five cases there were six deaths. The pressures were not low in these cases. He separated the recoveries into several groups, that is: In six cases there was no effect on pressure. In nine cases the curve showed a gradual progressive fall from 25 to 35 mm. which reached its lowest point, on the average, on the ninth day after the crisis and was unaffected by the crisis. In three cases there were steep falls which bore no relation to the crisis. In the twelve remaining cases the curve showed a progressive rise. In several instances the pressure rose 40 mm. during the crisis. Weigert found that he could not explain these enormous variations on the basis of age, sex, general strength of patient, severity or length of the disease, the extent of dyspnea, the kind or the amount of stimulation. His general conclusion is that one can establish no rule for the blood-pressure curve in pneumonia and that, consequently, blood-pressure readings in pneumonia are of no prognostic value.

In contrast to this view is the statement of G. A. Gibson,[3] that "a pressure appreciably below the normal in pneumonia is invariably of evil omen and any considerable fall bodes disaster. When the arterial pressure, expressed in mm. Hg does not fall below the pulse-rate, expressed in beats per minute, the fact may be taken as of excellent augury, while the converse is equally true. From the work of the last few years in my own wards no fact is more certain than this." This is known as Gibson's rule.

Gordon[4] confirmed Gibson's statement on the basis of observations of fifteen cases. Unfortunately the data from which Gordon's conclusions are drawn are omitted from his paper.

The blood-pressure in fifteen cases of lobar pneumonia occurring on the East Medical Service of the Massachusetts General Hospital from March 1, 1913, to Dec. 1, 1913, have been studied. The measurements have, as a rule, been made in the middle of the morning and the middle of the afternoon on each patient from entrance until death, or, in the case of recovery, well into the convalescence. All cases in which there was any doubt about the diagnosis and all persons who had any form of chronic cardiovascular disease were excluded. Further than this, there was no selection.

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3. Gibson, G. A.: Some Lessons from the Study of the Arterial Pressure, Edinburgh Med. Jour., 1908, n. s., xxiii, 17.

4. That point at which the fourth tone phase passed into the fifth was called the diastolic pressure.

5. Gordon, G. A.: Some Remarks on the Value of Accurate Blood-Pressure Estimation, Edinburgh Med. Jour., n. s., iv, 31.

All measurements were made by one of us (G. R. M.). The apparatus employed consisted of a graduated U-tube containing mercury attached to a broad arm-band. All the readings were made by the auscultatory method. Not only the systolic has been measured, but also the diastolic[4] and pulse-pressure. It was hoped in this way to gain more information than could be obtained by studying merely the systolic pressure.

There were five deaths in the group of fifteen cases. The cases of recovery were roughly divided into two groups, mild and severe according to the general clinical impression they made, regardless of the blood-pressure.

Figure 1 presents a summary of the blood-pressure data. The abscissae represent blood-pressure in millimeters of mercury. The space between each two ordinates represents a day of twenty-four hours. The last day on the chart is the day of the crisis or the day of death as the case may be. The upper and lower unbroken lines are the average of the systolic and diastolic pressures in the fatal cases. The averages were calculated in each case by placing all the measurements obtained during the last day of life in the last column on the right of the chart; the measurements of the next to the last day of life in the next to last column of the chart and so on until we had reached the day of entrance. In this way composite data for similar periods of the disease are obtained. The individual measurements for the fatal cases are indicated by X. The heavy broken lines represent the averages of the systolic and diastolic pressures of the persons who recovered. In this group all the measurements obtained on the critical day were placed in the last column of the chart, and all measurements obtained on the day preceding the crisis in the next to last column of the chart. The individual measurements are indicated by dots and the outer limits of the field by the fine broken line.

It is apparent that the systolic pressure in the fatal cases was continuously above the systolic pressure of the persons who recovered. We are, consequently, unable to accept Gibson's dictum that "a pressure appreciably below the normal is invariably of evil omen." It will be noted that the diastolic pressure of the recoveries shows a slight but progressive fall while the systolic pressure remains very nearly level. This results in an increasing pulse-pressure as the crisis is approached. The fatal cases show a slight tendency in the opposite direction. But the difference is small. By consulting the accompanying table it will be seen that the average of the pulse-pressures on the day of death in the fatal cases is 6 mm. less than the average of the pulse-pressures of the recoveries on the critical day.

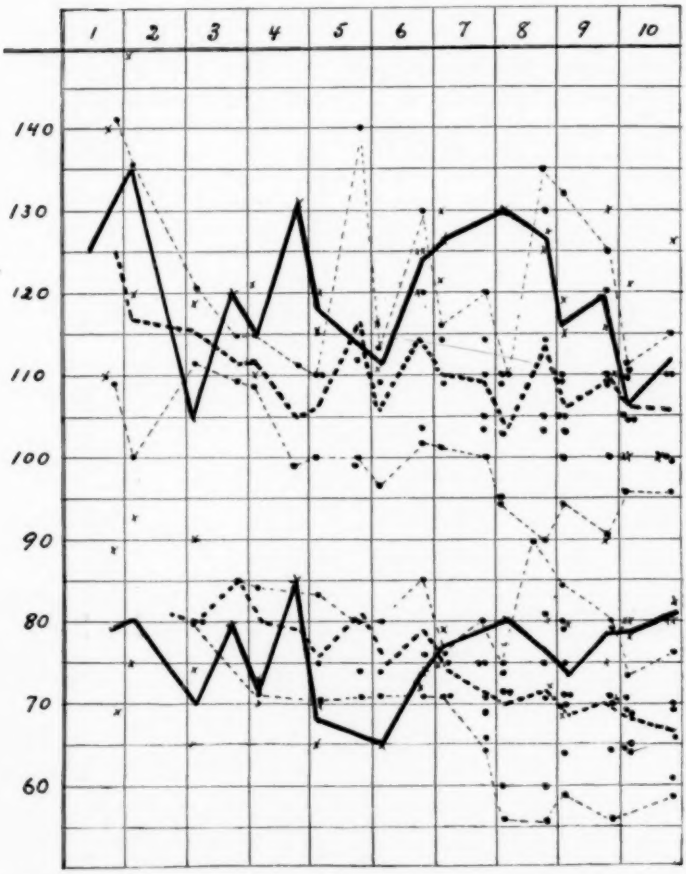

Fig. 1.—Presents a summary of the blood-pressure data from Massachusetts General Hospital cases. The abscissae represent blood-pressure in millimeters of mercury. The space between each two ordinates represents a day of twenty-four hours. The last day on the chart is the day of the crisis or the day of death as the case may be. The upper and lower unbroken lines are the average of the systolic and diastolic pressures in the fatal cases. The averages were calculated by placing all the measurements obtained during the last day of life in each case in the last column on the right of the chart; the measurements of the next to last day of life in the next to last column of the chart and so on until we had reached the day of entrance. In this way composite data for similar periods of the disease are obtained. The individual measurements for the fatal cases are indicated by X. The heavy broken lines represent the averages of the systolic and diastolic pressures of the persons who recovered. In this group all the measurements obtained on the critical day were placed in the last column of the chart. All measurements obtained on the day preceding the crisis were placed in the next to last column of the chart. The individual measurements are indicated by dots and the outer limits of the field by the fine broken line.

The table shows further that while the smallest pulse-pressures—20 mm.—occurred in two fatal cases, the largest pulse-pressure of the series was found also in a fatal case.

In Figure 2, two charts of temperature, pulse and respiration are reproduced to show that death may occur with a small or a large pulse-pressure, with a rising or a falling systolic pressure, with a systolic pressure above or below the pulse.

THE BLOOD-PRESSURE IN PNEUMONIA: READINGS ON DAY OF CRISIS OR DAY OF DEATH

Type	Systolic Pressure	Diastolic Pressure	Pulse-Pressure
Fatal	120–125	70	50
Fatal	125	80	45
Fatal	100	80	20
Fatal	100	80	20
Fatal	105	70–75	30–35
Average	110	77	34
Severe	120	80	40
Severe	110–125	70–80	40–50
Severe	110	70	40
Average	116	75	42
Mild	100	70	30
Mild	110	70	40
Mild	110	80	30
Mild	105	65	40
Mild	100	60	40
Mild	115	70	45
Mild	102	63	40
Average	106	68	38

Gibson has stated that "when the arterial pressure, expressed in mm. Hg, does not fall below the pulse-rate expressed in beats per minute, the fact may be taken as of excellent augury, while the converse is equally true." An analysis of the cases fails to justify this statement. Among five fatal cases the systolic pressure fell above the pulse twice—an error of 40 per cent. Among ten recoveries the systolic pressure failed to fall above the pulse five times—an error of 50 per cent.

Owing to the great kindness of Dr. L. F. Barker and Dr. W. S. Thayer, we have had access to the records of cases of pneumonia treated at the Johns Hopkins Hospital. For purposes of study the last fourteen deaths and the last sixteen recoveries from lobar pneumonia have been selected. Cases of children, all cases in which the

records were incomplete and also all in which there was any doubt about the diagnosis have been excluded. The pressure was measured in a routine way, two or more times daily by a number of persons; but, as a rule, the same person made all or nearly all the measurements on any single case. Systolic pressure only was recorded.

The course of the systolic pressure in the fatal cases has been compared with that of the persons who recovered, using the same method

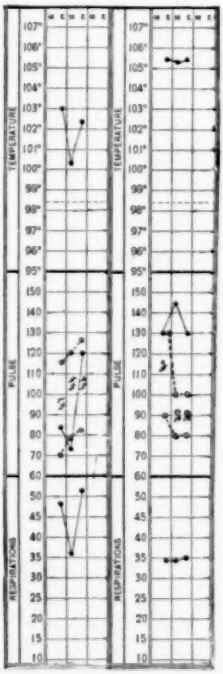

Fig. 2.—Charts of temperature, pulse and respiration in two fatal cases of pneumonia, showing that death may occur with a small or a large pulse-pressure and with a rising or falling systolic pressure, with a systolic pressure above or below the pulse. The lines of circles and dashes represent systolic and diastolic blood-pressure respectively.

as that employed for making Figure 1. Figure 3 shows the two curves. It will be noted that the curves of systolic pressure in both Figure 1 and Figure 3 lead to the same conclusions, that the systolic pressure in fatal cases tends to be higher than it is in the recoveries.

The working of Gibson's rule in these Johns Hopkins cases is as follows:

Among 14 fatal cases, 6 followed the rule, 4 were doubtful, 4 did not follow the rule, that is, the rule held 6 times out of 14 (43 per cent.).

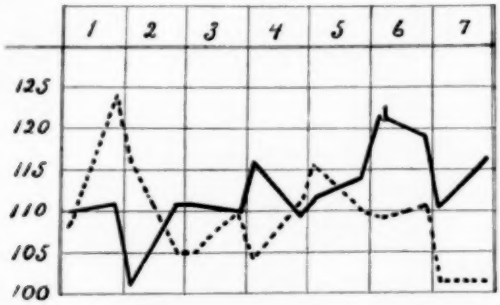

Fig. 3.—Curves of systolic pressure of fourteen fatal cases of pneumonia compared with sixteen recoveries taken from the records of the Johns Hopkins Hospital. Figure 3 was constructed in the same way as Figure 1.

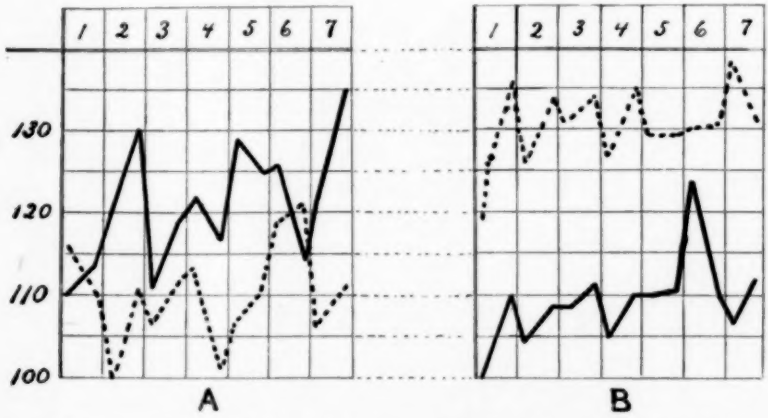

Fig. 4.—A: The solid line is the curve of systolic pressure in six fatal cases of pneumonia in which the systolic pressure was above the pulse. The broken line is the curve of systolic pressure in eight fatal cases in which the systolic pressure was below the pulse. B: The solid line represents the pulse rate in the same six fatal cases in which the systolic pressure is represented in A. The broken line is the pulse rate in eight fatal cases in which the systolic pressure is represented by the broken line in A.

Among 16 recoveries 6 followed the rule, 2 were doubtful, 8 did not follow the rule, that is, the rule held 6 times out of 16 (37 per cent.).

In both groups the rule held only in a minority of the cases and was definitely wrong 40 per cent of the time.

Lastly all the fatal cases (those of the Johns Hopkins Hospital and those of the Massachusetts General Hospital) have been divided into two groups with regard to the relation of the systolic pressure to the pulse. The first group includes the cases in which the systolic pressure expressed in millimeters of mercury fell above the pulse expressed in beats per minute. The second group includes the cases in which the systolic pressure fell below the pulse. In the first group there are eight cases, in the second, eight cases.

Figure 4 shows the relation of the pressures and the pulses in the two groups. It will be seen that the pressure curves fall very much closer together than the pulse curves. This immediately suggests that the rate of the pulse and not the level of the pressure, is the chief factor in deciding whether the pressure will fall above or below the pulse. That such is actually the fact can easily be determined by comparing the averages of all the measurements used in constructing each curve. By so doing, it is found that the average of the systolic pressures for the first group is 121, for the second group, 109, a difference of 11; whereas the averages for the pulses in the first group and second group are respectively 131 and 109, a difference of 22. The rate of the pulse is exactly twice as important as the height of the systolic pressure in determining whether the latter shall fall above the former or vice versa.

SUMMARY

1. The blood-pressure curve does not suggest that there is a failure of the vasomotor center in pneumonia.

2. Low systolic pressures are not "invariably of evil omen."

3. The systolic pressure in these fatal cases tends to be higher than it is in the recoveries.

4. Blood-pressure measurements in pneumonia cannot be used as a basis for treatment.

5. Prognostic inferences based on the relation of the level of the systolic pressure curve to the pulse curve (Gibson's rule) are wrong more often than they are right in this series.

6. The rate of the pulse and not the level of the pressure is the chief factor in deciding whether the pressure will fall above or below the pulse.

7. The pulse-pressure data are insufficient in number to justify conclusions.

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PNEUMOCOCCUS INFECTION AND LOBAR PNEUMONIA*

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Pneumonia, in many respects, certainly as a cause of death, is the most important infectious disease with which we have to deal. The symptomatic treatment is difficult and of doubtful utility; there is no well-established form of specific therapy. Nevertheless, up to within a very recent time, the investigation of the real nature of the process has been slight and fragmentary.

While the association of certain kinds of bacteria with this disease has been well established, much obscurity exists with regard to the mode of infection, the relation of the bacteria to the lesions and symptoms, the nature of recovery and, above all, with regard to the possibility of prevention or of cure. It has been in the hope of helping to shed some light on these problems that my associates and I have been making some clinical and experimental studies. It will be impossible to review in this paper all the work that has been done by others, and I shall have to content myself with presenting certain points of view which have been suggested mainly by our own work at the Rockefeller Institute.

Acute lobar pneumonia seems the best characterized of the acute lung affections. It has such a clear-cut, clinical course that it is now generally considered a distinct clinical entity, and no more to be regarded simply as an infection of the lung than typhoid fever is to be considered an infection of the intestine. This, however, is a clinical point of view and it is possible that the same kinds of reaction occur in other localized pneumococcus infections as are present when the main seat of infection is the lung.

In a very large percentage of patients suffering from inflammation of the lung of the lobar type, pneumococci are present in the lesion. In isolated instances, other organisms, as *Bacillus influenzae* or *B. pneumoniae*, are found in pure culture. It has not been our purpose to consider these latter cases, but our attention has been given entirely to the group of cases in which *Diplococcus pneumoniae* is present and is apparently the etiological agent. As is well known, diplococci, which

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at present cannot be differentiated from the pneumococcus, may be present in pneumonia of the lobular tube (in which the clinical course is quite distinct from that in lobar pneumonia); they may also be present in other purely localized lesions in the body, entirely unassociated with any affection of the lung, and they may even be the organisms concerned in certain cases of septicemia in man without any local lesions whatever. Moreover, organisms with identical characteristics, so far as yet determined, are found with so great frequency living on the mucous membranes of the mouth and throat of perfectly healthy individuals that they may be considered normal inhabitants of the mouth and throat cavities. In the face of such facts as these, how can it be maintained that *Diplococcus pneumoniae* is the primary cause of such a well-characterized acute infectious disease as acute lobar pneumonia? In view of the present general consensus of opinion that this theory is true, one is indeed rash even to suggest the possibility that there may be another agent concerned. On the other hand, it is important that such a possibility should not be overlooked. Even though it should be shown, however, that pneumococci do not play the primary etiologic rôle in the natural infection, their association with the lesion and their frequent invasion of the blood render it evident that they play an important part in the process and probably the most important part in the outcome, just as do streptococci in certain diseases, such as small-pox and scarlet fever, of which it is generally believed that the natural infection is due to specific etiologic agents.

Up to within a relatively short time, the most important link in the chain of evidence that pneumococci cause pneumonia, namely the reproduction of the disease in animals, was lacking. Most important studies dealing with the experimental production of acute lobar pneumonia were published in 1904 by Wadsworth.[1] By carefully balancing the general resistance of the animal with the virulence of the race of pneumococci employed and by injecting the organisms intratracheally, he was able, in a series of rabbits, to induce a diffuse exudative pneumonia like the acute lobar pneumonia seen in man.

More recently Lamar and Meltzer,[2] and Wollstein and Meltzer[3] have succeeded in regularly producing a diffuse pneumonia of the lobar type in dogs, by injecting from 10 to 15 c.c. of the fluid culture directly into one bronchus through a rubber tube passed through the trachea, following the injection of the fluid by air blown through the tube, so as to force the infectious material into the finer ramifications of the

1. Wadsworth: Am. Jour. Med. Sc., 1904, cxxvii, 851.
2. Lamar and Meltzer: Jour. Exper. Med., 1912, xv, 133.
3. Wollstein and Meltzer: Jour. Exper. Med., 1913, xvii, 353, 424.

bronchi. The pneumonia produced in dogs runs a more rapid course, resolution occurs earlier—in three or four days—and the mortality is much less than in pneumonia in man.

Using a similar technic, these investigators have produced diffuse lesions in the lungs of dogs with other micro-organisms. When streptococci are injected, the lesions tend to resemble more closely those seen in bronchopneumonia in man.[4] The observers lay stress on the greater tendency in this case to a leukocytic infiltration of the lung framework, and to a much-lessened formation of fibrin. These differences between the pneumonia produced by the injection of streptococci and that following the injection of pneumococci they ascribe to inherent differences in the nature of the micro-organisms concerned, and not to relative differences in virulence. By a similar method of intratracheal injection, Winternitz and Hirschfelder[5] have succeeded in producing pneumonia of a lobar type in rabbits. In these experiments also, large amounts of the culture material (4 or 5 c.c.) were injected.

From experiments which I have carried on, using the same method, it is evident that successful results in rabbits depend somewhat on the race of organisms employed. If the organisms have very slight virulence, the animals may recover without lung lesions; if they are too virulent, a septicemia quickly results and at necropsy only congestion and edema of the lungs are present.

As Wadsworth showed, the lung consolidation is probably a manifestation of the resistance of the animal to the spread of the infection. The occurrence of the diffuse lung lesion is undoubtedly dependent on the same factors which are concerned in the differences in local reaction to the injection of virulent pneumococci in different races of animals. As is well known, mice and rabbits are very susceptible to pneumococcus infection, however induced, guinea-pigs and dogs less and man possibly still less. The result of a subcutaneous injection of virulent pneumococci into a rabbit differs markedly from that seen when a similar injection is made into a guinea-pig. In the former there is very little local reaction; a rapid general invasion of the organisms takes place, and the animal dies quickly from a marked septicemia. In the latter a marked infiltration with much fibrin formation and a slowly progressive invasion of the subcutaneous tissues occur, while there is little or no general infection. That the time element plays a rôle in the formation of fibrin is well seen in the peritonitis induced in such susceptible animals as the mouse and rabbit. If an intraperitoneal injection is made of virulent pneumococci, death occurs within twenty-four hours, and in the peritoneum there is seen only

4. Wollstein and Meltzer: Jour. Exper. Med., 1913, xviii, 548.
5. Winternitz and Hirschfelder: Jour. Exper. Med., 1912, xvii, 657.

a marked congestion, possibly hemorrhages, a serous exudation and usually no fibrin. If, however, the culture is less virulent and the animal lives forty-eight hours or more, there is usually considerable fibrin over the liver, and flakes of fibrin are seen throughout the cavity. The amount of fibrin increases in ratio with the length of time during which the animal is able to resist the infection.

The experiments of Wollstein and Meltzer,[4] however, tending to show that the peculiar property of stimulating the production of fibrin is possessed to a greater degree by pneumococci, whatever their virulence, than by streptococci, is of very great significance. It is difficult to understand, however, just why this property should be a factor in the production of pneumonia of a lobar rather than of a lobular type. That this peculiar property should not be the only one concerned is made evident from the fact that the pneumococcus is the organism most frequently concerned in lobular pneumonia in children. Dochez[6] has shown that, during the acute stages of lobar pneumonia, there is an increase of fibrinogen in the blood. Nevertheless, the coagulation time of the blood is delayed, owing probably to an increase in antithrombin as well. We know of no observations which show whether or not so great an amount of fibrinogen exists in the blood in prolonged streptococcus infections in man as is present in pneumonia.

As the virulence of the race employed is of importance as regards the production of the local reaction, so also is the number of microorganisms injected, as Kline and Winternitz[7] have shown. If the number of organisms is too small, no pneumonia results. It is well known that even in very susceptible animals a considerable number of virulent organisms is usually necessary to produce infection. Little attention has been given to the question why, when a considerable number of organisms are injected together, multiplication occurs and infection results, whereas if only a few organisms be injected, they are unable to multiply. Is it because in a culture of organisms certain ones are more resistant than others to the harmful influences, or is it simply accidental that, when a large number of organisms are injected, a few have a possible chance to escape?

Gillespie[8] has carried on some experiments with pneumococci which have a bearing on this problem. We, as well as others, have long recognized that in starting a culture of pneumococci in a large amount of bouillon, a liter for instance, a much larger inoculation is necessary in order to obtain growth than if the inoculation be made into 10 c.c., or if the culture be made on a solid medium. In the latter instance,

6. Dochez: Jour. Exper. Med., 1912, xvi, 693.
7. Kline and Winternitz: Jour. Exper. Med., 1913, xviii, 50.
8. Gillespie: Jour. Exper. Med., 1913, xviii, 584.

one organism will usually produce a colony. By making the inoculations on filter-paper kept constantly wet by bouillon, it was shown that growth would occur with the inoculation of as small numbers of organisms as are required on agar, and with much smaller numbers than are required to inoculate the bouillon. The differences in growth, therefore, are not dependent on differences in composition of the medium, and further experiments have shown that they are not due to differences in oxygen supply. It seems probable that for growth to occur, the bacterium must produce changes in the medium immediately surrounding it, and that when the opportunities for diffusion are great, such local changes cannot be kept sufficiently constant unless there be a considerable number of organisms in proximity. If this be the true explanation, it may have an important bearing on infection, not only with pneumococcus but with other micro-organisms as well. The presence of mucus in the smaller bronchi, for instance, might in this way favor the multiplication of micro-organisms and so favor infection. That very large numbers of bacteria are inoculated in the experiments of Meltzer and Winternitz probably explains to some degree why their experiments have been successful, where others have failed. A second factor in the success of this technic probably lies in the fact that considerable amounts of fluid are injected and this is blown into the terminal bronchioles. Meltzer has made the interesting suggestion that by this process the bronchioles are occluded and that in this way closed cavities are formed. It is generally recognized that as long as bacteria grow on exposed surfaces, they do no harm. It is only when the growth occurs in confined spaces that harmful results supervene.

While these experiments on the production of pneumonia in animals are of great value in showing that lesions resembling acute lobar pneumonia in man may be caused by pneumococci, they do not directly offer an explanation of the natural infection in man. It is hardly likely that in man an overwhelming infection ever occurs with numbers of bacteria so large as those used in the experiments in dogs. The usual recourse in this dilemma is to assume that the organisms concerned in natural infection have an increased virulence, or that the resistance of the host is lowered. By virulence in micro-organisms is usually meant adaptation to growth in the tissues of the host. Since in pneumonia the organisms are cultivated from the lungs and at times from the blood, we know that they have virulence for man. We have no way of determining, however, whether or not all pneumococci growing in the mouths of healthy individuals are also virulent for man. The attempts to demonstrate conclusively that pneumococci isolated from cases of pneumonia in man are regularly of increased virulence for animals have not been successful. We have found that most of the

pneumococci isolated from the blood of pneumonia patients have relatively high virulence for susceptible animals, yet while certain of these cultures, when freshly isolated, are of such virulence that 0.000001 c.c. of a bouillon culture will kill a mouse, we have also obtained cultures that required 0.5 c.c. to kill. The virulence of the race, therefore, does not seem to be the only deciding factor in the question why infection occurs, even though, as will be shown, it may be of considerable importance as regards the final outcome. It must be stated, however, that it is not certain that virulence for animals is identical with virulence for man. Usually high virulence of a given race of pneumococci for one susceptible animal, as the rabbit, indicates high virulence for another, as the mouse. Ungermann, however, has described a typical pneumococcus having high virulence for rabbits, but its virulence for mice, which was originally present, was lost. We have studied a race of pneumococci originally very virulent for mice, which, after passage through guinea-pigs, increased its virulence for these animals, but became almost avirulent for mice and rabbits.

Against the view that pneumonia arises when organisms of increased virulence reach the lung is the fact that pneumonia rarely occurs in epidemics and is very slightly contagious. There are now a number of epidemic outbreaks reported, but it must be admitted that these are of rare occurrence, and all hospital experience is against contagion as a great factor in the spread of the disease. On the other hand, the fact that in certain times and places pneumonia occurs with greatly increased severity and frequency suggests that at these times the pneumococci concerned may have acquired increased virulence. When the French attempted to build the Panama Canal, the incidence and mortality of pneumonia interfered as seriously with the work as did the occurrence of malaria and yellow fever. Even during the first years of the American occupation of Panama, the mortality from pneumonia was enormous. For its decrease there seems no adequate explanation. In South Africa, deaths from lobar pneumonia among the coolies constitutes a serious menace to the continued working of the mines.

Little is known concerning diminished general resistance on the part of man to pneumococcus infection and its importance in natural infection of this disease. Clinical studies have quite conclusively demonstrated that the habitual use of alcohol increases susceptibility to infection or, at any rate, renders the subject less resistant when infection has once occurred. That exposure to cold, or especially sudden changes in temperature and chilling, play a part in infection can hardly be doubted, and there is some experimental evidence to show that animals suddenly chilled are more susceptible to infection with pneumococci than others.

The view that local changes in the lung are of importance for the occurrence of infection is interesting and suggestive. In most cases of lobar pneumonia the primary seat of infection is probably in the lung. Various writers have attempted to show that infection occurs through the blood-stream, but the evidence is not conclusive. Other localized pneumococcus infections in internal organs or cavities usually occur by extension, though this is not always demonstrable. The possibility that infection through the blood may occur in certain instances cannot be excluded. Pneumonia, as a part of a general infection, however, is generally of a lobular type.

Much has been said lately about the adaptation of certain organisms for certain tissues. In many cases, however, this adaptation is more apparent than real, and the mode and degree of infection play the larger rôle in localization.

In about 50 per cent. of the cases of pneumonia, a history of preceding coryza and cough may be obtained. In these cases it is possible that there occurs a downward infection along the mucous membrane of the bronchi. The extension of the lesion through the lung from one lobe to another apparently takes place through the bronchi, as the study of large sections through lobes with beginning involvement shows. In the remaining half of the cases, however, the onset is sharp and sudden without history of bronchial involvement. Even in these cases some local change probably precedes the real infection. It is well known that thoracic trauma is frequently followed by pneumonia. The idea of Meltzer that infection may be facilitated by closure of the smaller bronchioles is most suggestive. It is possible that cold or chilling may stimulate the mucous glands so that the increase of mucus may produce favorable conditions for the growth of pneumococci, which are so frequently present in the upper respiratory tract.

That the lung is the chief seat of the disease, then, is probably due to the fact that infection occurs here, and that a local lesion results and not a general infection (at least not until late in the disease), is probably due to the fact that man is highly resistant to infection with pneumococci and that the anatomical conditions here permit of an extensive inflammatory reaction which opposes the spread of the infection.

But why does infection occur at all? Why does a person contract pneumonia? There is still considerable obscurity in regard to this phase of infection, not only in pneumonia, but also in many other infectious diseases of which the etiology is well known. The obscurity and difference of opinion in regard to tuberculosis is well known. Even our views with regard to infection in diphtheria have been disturbed

by new observations. We all thought the transference of the infectious agent from the infected to the healthy throat was all that was necessary for infection in this disease. The observations of Moss, Guthrie and Gelien,[9] however, that in Baltimore there are four times as many carriers of virulent diphtheria bacilli as there are cases of the disease, are most disturbing. So far as can be determined, the bacilli from carriers differ in no way from the bacilli from patients with the disease. Moreover, the incidence of the disease seems to bear no relation to these carriers. The problem of mode of infection is thus in a minor degree analogous to that in pneumonia, in which practically the entire population represent carriers of infection.

To explain the nature of infection we may say: First, there is a possibility that in pneumonia, as in diphtheria, the organisms causing infection differ inherently from those in normal throats, especially as regards adaptation or virulence for men. Definite evidence in favor of this would be most important, but at present there is none. Second, it is possible that the general resistance of patients to pneumococci is lowered, so that organisms, formerly living as harmless parasites, now invade the tissues and induce reaction. For this also we have no definite evidence. Third, the study of artificial infection in animals, as well as the course of the disease in man, suggests most strongly that local changes in the respiratory tract may precede the infection with pneumococci. Whether these are due to a primary infectious agent or to changes in the tissues due to other factors cannot be decided definitely at present. Finally, it is possible that infection depends on a combination of factors, virulence of organisms and general and local resistance each playing a part. Further knowledge along these lines is absolutely essential for prevention of this disease. To formulate rules or regulations for prevention at present seems useless, except as an experiment.

THE NATURE OF THE INTOXICATION

Whatever be the mode of onset in pneumonia, the production of the local changes in the lungs, as well as the general systemic manifestations of the disease, seems to be in some way related to the growth of pneumococci in the body. When micro-organisms grow within other multicellular organisms acting as host, the effects on the host are of two kinds: First, there is a local reaction, in which the bacteria are present in considerable numbers, as at the point of infection. Here are induced the changes spoken of as inflammation. In addition to this, however, there is practically always a reaction throughout the entire

9. Moss, Guthrie and Gelien: Tr. Fifteenth Internat. Cong. on Hyg. and Demog., Washington, 1912, iv, 156.

body, even when the local reaction is very mild and evanescent. These general reactions are evidenced, not only by fever and nervous disturbances on the part of the host, but even in their absence by such effects as changes in the blood, especially the leukocytes, which indicate certain effects on the blood-forming tissues.

In most cases, the exact manner in which micro-organisms stimulate the tissues in which they are growing to a reaction which is called inflammatory, is still obscure. Since, however, identical reactions may be produced with non-living chemical substances, it is generally assumed that in the case of bacteria as well, non-living chemical substances are formed as a result of the bacterial growth, which substances are in themselves harmful. Whatever may be the exact relationship of the organisms to the local lesion, it is necessary to assume that the general manifestations of infection, and especially the effects on tissues far distant from the local lesion, are the result of soluble toxic substances which circulate in the blood or lymph. Since general manifestations similar to those in pneumonia are seen in other pneumococcus infections and even in general infections in animals without local lesions, it does not seem probable that these effects in pneumonia are due to disturbances in respiration associated directly with the lung lesions. It is possible, of course, that in pneumonia the general manifestations and effects on distant tissues and organs are due to the action *in situ* of bacteria which have gained access to the circulation and have been carried to these distant parts.

Very numerous observations have been made on the occurrence of bacteria in the general circulation in acute lobar pneumonia. During the past years blood-cultures have been made on most of the cases of pneumonia coming under my observation, and the results have not led me to change the conclusions arrived at ten years ago from the study of a series of cases, namely, that pneumococci are usually found in the blood only in the more severe cases, and the presence of the pneumococcus in the blood is of ill omen. It is possible that in all cases of pneumonia an occasional bacterium may be carried into the circulation, but the demonstration of this is difficult. That this may occur, however, is not of prime importance, for the occurrence of an occasional organism could hardly explain the great degree of effect in distant tissues, as manifested by the general symptoms which we call intoxication.

The attempts to discover something of the nature of this circulating poison have been attended with much difficulty. It would seem that a more accurate knowledge of the metabolic disturbances in pneumonia might give a clue as to the nature of the intoxication. A series of studies with this object in view was undertaken.

Of late years attention has been drawn to the occurrence of functional disturbances, especially in infants, due to derangements in salt metabolism. It appeared of interest to learn whether or not specific changes in inorganic metabolism may be induced by pneumococci, which could account, in part at least, for the symptoms induced. The most striking disturbance in pneumonia is known to be the retention of chlorids, which is frequently almost complete during the acute course of the disease Retention of chlorids to a lesser degree is known to occur in other infections, but Rowntree[10] has shown that this retention does not occur in influenzal pneumonia to nearly so marked a degree as it does in pneumococcus pneumonia. Medigreceanu[11] has carried on a series of studies of pneumonia in dogs and Peabody[12] has studied the question in cases in man. Peabody has shown that there is a retention not only of chlorin, but also of sodium and calcium while there is no retention of potassium and magnesium, but may be a loss. Further studies indicate that the retained substances are not stored in any one place, but are spread diffusely throughout the tissues.

It is not believed that these changes are specific for pneumonia, for they probably occur in other infections. They are most striking in pneumonia, since the changes between the febrile and afebrile state occur with such suddenness. It is not likely that these changes in themselves are responsible for any of the symptoms of the disease, but in view of the striking effects which have been induced by Meltzer by changing the balance in the inorganic salts in the body, this possibility must be borne in mind. We have no knowledge of the reason for these changes in pneumococcus infection.

Pneumococci are known to produce acid readily, even, as shown by Hiss, in albuminous mediums, containing no demonstrable sugar. It has therefore been suggested that the symptoms in pneumonia are the manifestations of an acidosis. Hamburger[13] has attempted to explain the chlorin retention on the basis of a febrile acidosis. The studies of Peabody,[14] however, have shown that the curves of chlorin retention and of ammonia excretion, which is generally considered the best indicator of acidosis, do not necessarily run parallel. The studies of inorganic metabolism have therefore given no conclusive insight into the nature of the intoxication.

In order, if possible, to obtain some knowledge regarding this problem by another method, Peabody has made studies of the gas exchange

10. Rowntree: Bull. Johns Hopkins Hosp., 1908, xix, 367.
11. Medigreceanu: Jour. Exper. Med., 1911, xiv, 289.
12. Peabody: Jour. Exper. Med., 1913, xvii, 71.
13. Hamburger, H. J.: Osmotischer, Druck und Ionenlehre, Berlin, 1912.
14. Peabody: Jour. Exper. Med., 1912, xvi, 701.

in the blood in pneumonia. He has found that in this disease the carbon dioxid in the venous blood is quite regularly low, in spite of the disturbances in gas exchange in the lung. At the same time there occurs an increase in the ammonia nitrogen in the urine, and the curves run somewhat parallel. These changes, which are indicative of increased acid formation, nevertheless correspond to changes that have been known to occur during fever and infection due to other causes, and are no indication of specific changes occurring in this disease. The carbon dioxid content of the blood does not bear a definite relationship to the severity of the disease, except that it is lowest in the most severe cases and in the terminal stages.

On the other hand, the study of the oxygen-content of the blood has revealed some interesting changes. Studies of the peripheral venous blood showed in certain cases a diminution in the oxygen-content of the venous blood. In studying the blood in one such case, it was found that the blood would not take up a normal amount of oxygen, and this in spite of the fact that the hemoglobin content was normal. In a careful study of such blood by Butterfield and Peabody[15] it was found that this phenomenon was due to the formation of methemoglobin. This change also occurs regularly in the blood of rabbits[16] infected with the pneumococcus and has no relation to the lung lesion. It also occurs when the bacteria are grown in blood-containing mediums.

Usually the change into methemoglobin in the animal body does not go so far that the methemoglobin can be distinguished spectroscopically. In the test-tube, however, especially when hemoglobin in solution is added to the culture, practically all the hemoglobin may be changed into methemoglobin. That this reaction is not simply due to the action of acids formed by the pneumococcus is shown by the fact that for the production of methemoglobin far more acid is required than could be present in the body, and, moreover, that it may occur in cultures or filtrates that are alkaline in reaction. It is therefore evident that this change is due to the action of a poison formed by the pneumococci.

Peabody[17] further made a study of the blood in twenty-five cases of pneumonia to determine the frequency of the occurrence of this phenomenon and the time of its appearance. Of the cases which ended in recovery, in only one was there any indication of a diminution of the oxygen-absorbing power of the hemoglobin. In all of the ten cases ending fatally, there occurred a progressive loss in the oxygen content of the blood and in the oxygen-combining power of the hemo-

15. Butterfield and Peabody: Jour. Exper. Med., 1913, xvii, 587.
16. Peabody: Jour. Exper. Med., 1913, xviii, 1.
17. Peabody: Jour. Exper. Med., 1913, xviii, 7.

globin, and from the previous studies it is certain that these changes are due to the formation of methemoglobin. In nine of the ten cases the blood-cultures were positive.

That these changes play a part in the fatal termination can hardly be doubted. The terminal symptoms of the disease may be accounted for by deficient oxidation. It is not likely, however, that these changes in the blood are in themselves the only factor in accounting for the fatal result; but they represent one of the factors, and are an indication of the intoxication which is the result of the growth of pneumococci in the body.

A second effect of the pneumococcus intoxication has been demonstrated by Medigreceanu† by estimating the amount of oxydase in the organs of animals dying from pneumococcus septicemia, as compared with the organs of normal rabbits. In these studies Medigreceanu employed the method of Röhman and Spitzer, which is based on the property of tissues of oxidizing a mixture of naphthol and paraphenylendiamin into phenol. By comparing the tissues of normal animals with those previously infected with pneumococci, it has been found that this oxydase is generally diminished in the latter animals. By proper controls it has been possible to show that this change is due, not to the presence of pneumococci in the tests, but to some change which results in the tissue from the infection. Another effect of the action of the toxin on tissue function is thus made evident. It is therefore probable that, in addition to the lessened supply of oxygen by the blood due to the formation of methemoglobin, there is also a lessened power of the tissues to carry on the proper oxidation function.

Finally, in order to obtain evidence of the presence of a poison, studies were made by Medigreceanu[18] to determine whether or not there was an increased output of substances known to have the property of neutralizing poisons arising in the body. Such a substance is glycuronic acid, and it was found that during the acute stages of pneumonia in man, in almost all cases, there is a definite increase in the output of this substance.

All these studies clearly indicate the activity in pneumonia of a circulating poison; but the direct demonstration of the presence of this toxic substance in the animal is more difficult. To this end the following experiments were performed. Each one of a series of rabbits was inoculated with an overwhelming dose of pneumococci. Then, just as death was imminent in from five to eight hours, the animal was bled to death, and as quickly as possible the blood was defibrinated, the serum passed through a Berkefeld filter to remove the bacteria and

† Medigreceanu: Jour. Exper. Med., 1914, xix, 309.

18. Medigreceanu: Jour. Exper. Med., 1913, xviii, 259.

the filtrate injected intravenously into a normal rabbit. To our surprise and disappointment, the animals did not die, nor in a second series of rabbits treated in this way were we able to detect any minor harmful effects of such injections.

When one considers the conditions in pneumococcus infection it is not surprising that there is great difficulty in demonstrating the presence of toxin in the animal, or even of demonstrating the production of toxin by the pneumococcus *in vitro*. The infectious diseases in which active specific toxins have been well demonstrated *in vitro* are diphtheria and tetanus. In these diseases, however, the conditions are unusual. Here a moderate number of organisms growing in the local lesion produce sufficient poison to bring about the most profound intoxication, and it is not surprising that the poison may readily be demonstrated *in vivo* and *in vitro*. In pneumococcus infection, the conditions are different. Even in general infection in the highly susceptible mouse or rabbit, the number of organisms growing in the body is enormous before the animal finally succumbs.

In the severe and fatal cases in man the blood may contain as many as 65,000 organisms per cubic centimeter; and when it is conceived that these are throughout all the body-fluids and the tissues, it is evident what immense numbers of bacteria are responsible for the intoxication and fatal outcome. In man, even when there is no marked invasion of the blood, the number of micro-organisms in the lung must be very large. It is probable that during the course of the disease bacteria are all the time undergoing degeneration, so that from the beginning to the end, large numbers of bacteria have been present. Also the amount of toxin present at any one time may be very small, yet when acting on tissue-cells for six or seven days may produce marked effects.

The suggestion has been made that in pneumonia the symptoms are due to the absorption of products of digestion of the pathological exudate in the lung. It has been well established by various observers that, during the parenteral digestion of protein, substances are formed which may induce fever and symptoms of intoxication. Similar symptoms may be induced by the injection of peptone and other products of protein digestion, into the circulation of animals. Most of the work that has been done in the production of fever by means of protein, however, has been carried out with foreign protein and not with the protein of the host. Moreover, at the time the resolution is probably greatest, that is, following the crisis, such symptoms are not present, although in most cases considerable amounts of the digested exudate are being absorbed. It is hardly likely that the specific and characteristic symptoms of pneumonia can be due merely to the absorption of the products of digestion in the local lesions.

Following the discovery of serum anaphylaxis in guinea-pigs and its relation to protein intoxication in man, numerous efforts have been made to bring this into relation to the toxic effects seen in acute infectious disease. Friedberger has shown that if bacteria be treated with immune serum and then with complement, the supernatant fluid, after removal of the bacteria by centrifugalization, will be toxic when injected into a guinea-pig, the animal dying within a few minutes with symptoms identical with those seen in anaphylactic shock. He has called the toxic substance produced outside the body in the manner stated "anaphylatoxin," and believes that it is identical with the substance formed within the body which produces the symptoms following the second injection of protein. He thinks that this experiment may offer the explanation for all bacterial intoxications. According to his theory in the period of incubation, during which the bacteria are already present but produce no symptoms, antibodies are being formed, and when these are present in sufficient numbers, the bacterial bodies begin to be split up and the substances so formed produce, not the acute symptoms which are speedily followed by death, because the bacteria are not present in sufficient numbers, but milder symptoms —fever, etc., a kind of chronic anaphylaxis. This can hardly explain, however, what occurs in pneumonia, in which all the evidence seems opposed to a long incubation period, the onset of the symptoms being sudden and apparently the immediate result of the infection.

The production of the so-called "anaphylatoxin" from pneumococci may readily be done, as we and also Neufeld and Dold have shown. Neufeld and Dold,[19] moreover, have shown that similar toxic substances may be obtained from bacteria by simple extraction in salt solution containing lecithin. Rosenow[20] then showed that if pneumococci are merely placed in salt solution for forty-eight hours at 37 C. (98.6 F.), the extract so formed is toxic, and on injection intravenously into guinea-pigs, acute symptoms and speedy death, like those seen in serum anaphylaxis, result. We have studied the effect of the injection of extracts obtained by autolysis in a very large number of guinea-pigs,[21] and, in our experience, while occasionally sudden death is produced, this does not occur with great regularity.

Since the salt solution extracts of pneumococci did not show as high toxicity as was anticipated, it was held possible that in the peritoneal cavity of an animal the solution of the bacteria might go on at a more rapid rate, from which cavity solutions might be obtained of greater and more constant toxicity. Guinea-pigs were therefore inocu-

19. Neufeld and Dold: Berl. klin. Wchnschr., 1911, xlviii, 1069.
20. Rosenow: Jour. Infect. Dis., 1911, ix, 190.
21. Cole, Rufus: Jour. Exper. Med., 1912, xvi, 644.

lated intraperitoneally with large doses of pneumococci. As soon as possible after death, the peritoneal cavity was washed out with salt solution. The cells and bacteria were then removed from this solution by centrifugalization and the supernatant fluid was used for intravenous injection into healthy guinea-pigs. Of eleven animals so treated, eight showed immediate symptoms like those seen in anaphylaxis, and four of these died within a few minutes with typical features of anaphylactic death and with characteristic necropsy findings.

From the experiments it is evident that the development of the toxic substance is more constant and striking in the peritoneal cavity of the guinea-pig than it is in the test-tube. In the animal body, however, conditions are complex and it is difficult to know whether the toxic substance is specific or bears any direct relation to the infectious agent. We therefore tried to obtain solutions of the pneumococcus bodies by other means. Making extracts in chloroform and in ether did not yield solutions that could be readily studied. We next studied the solution of pneumococci obtained by means of bile. In making the solutions a 2 per cent. solution of sodium cholate in normal salt solution was employed. The effect of the intravenous injection of bile extracts of pneumococci has now been tested in a very large number of guinea-pigs and rabbits. In a large proportion of cases death with acute symptoms resembling those in anaphylactic shock occurs. When smaller amounts of the extract are injected, or when the toxicity of the extract is less, the animals die in from two to twelve hours. Such animals usually show more or less pulmonary edema and hemorrhages, and small hemorrhages are present in the peritoneum and diaphragm and in the walls of the stomach and intestines.

It is probable, from the effects produced, that the substances operative here are the ones that produce the effects in "anaphylatoxin" and in the salt solution extracts. In the latter case it has been assumed by Rosenow that the toxic substances result from the digestion of the bacterial protein by the ferments contained in the bacterial cell. The proof of this, however, does not seem convincing. The fact that the solution of the pneumococci in cholate solutions may occur within one-half hour at 4 C. (39.2 F.) is evidence that in this case the active substance is not the result of ferment action. In a recent communication Jobling and Strouse[22] have presented good evidence to show that the lysis of pneumococci in salt solution is probably not merely the result of ferment action. All these experiments indicate that the bodies of pneumococci contain substances which are toxic when they are set free by the solution of the bacterial bodies. They therefore present evidence in favor of the well-known endotoxin theory of Pfeiffer.

22. Jobling and Strouse: Jour. Exper. Med., 1913, xviii, 597.

During the past few years this theory of the origin of toxic substances has been largely neglected, owing to the interest in the theories of Vaughn and Friedberger, according to which the intoxication in all forms of infection is caused by substances which are intermediate products in the digestion of protein. Vaughn goes so far as to state that the substances producing the symptoms are identical in all infections and that the different symptom-complexes are dependent, not on the nature of the intoxicating substance, but on other conditions. It would hardly seem, however, that the intoxicating substance causing the rapid pulse and rapid, labored respiration and violent delirium of pneumonia is identical with the intoxicating substance in typhoid in which there is a relative slowing of the heart and low, muttering delirium. Though the intoxication may be due to the products of protein digestion, it does not necessarily follow that the substance is the same in all cases, as the bacterial proteins must differ enormously in composition.

While the obtaining of toxic substances from the bodies of pneumococci is of great interest, it is quite evident that this, in itself, does not contain the proof that we are dealing with the substances responsible for the intoxication in pneumonia. In order to present such evidence, further knowledge is required of the nature of the substance and especially of its relation to pneumococcus immunity.

Certain facts have already been established in regard to this toxin. Its study has been greatly facilitated by the fact that when added to washed sheep-corpuscles hemolysis occurs. So far as studied, the toxic effects are caused by the same substance which produces hemolysis, since the two properties are influenced by the same measures and vary in equal degree. One of the most important facts that has been determined in regard to this toxin is that the toxic and hemolytic properties vary with the virulence of the organism employed. Extracts from non-virulent cultures, so far as studied, are not toxic. The substance which is responsible for the formation of methemoglobin in the body and the discoloration of blood in cultures, however, does not seem to be present in this toxin. The toxin is destroyed by heating one-half hour at 56 C. (132.8 F.). This may explain why the injection of pneumococci killed by heat produces no effect in the animal injected. It loses its toxicity when kept for twenty-four hours at 37 C. or for two or three days on ice. It may be dried, in which condition its toxic properties disappear much more slowly. It does not pass readily through a Berkefeld filter and it is precipitated by colloidal iron solutions. Many attempts have been made to neutralize its action by the use of dyes, by nucleic acid, nucleates, glycocoll, glycuronic acid, etc., substances which are considered to attach themselves to toxic sub-

stances in the body and thus to render them non-toxic. None of these experiments have been successful. The only substance so far found which is able to neutralize the effect of the toxin is cholesterin. When cholesterin is mixed with the toxic substance and kept at 37 C. for fifteen minutes, the toxic effect, as shown by injection into animals, and also the hemolytic effect, is lost. When the toxin is mixed with cholesterin and immediately injected into the animal, however, or when the toxin is first injected and is immediately followed by the cholesterin, the toxic effects cannot be prevented. Nor can the toxic effects be prevented by injecting the cholesterin before the toxin. The most important results in this study have been obtained in the attempts to produce antiserums to these toxins, and of these I shall speak later.

Whatever may be the mechanism by which intoxication is brought about, have we any evidence as to the determining factors in the final outcome, that is, as to why the patient recovers or dies? The results of our blood-cultures would seem to indicate that the occurrence of septicemia plays an important part in the death of the patient. A study of the virulence of the cultures from the blood also seems to show that the intoxication is greater and the prognosis worse when the organisms have a high virulence than when they have a low virulence. Moreover, our clinical experiments seem to indicate that the progressive extension of the local lesion is of bad prognostic import; that the failure of the body to erect a limiting barrier to the local extension of the disease is an important factor in the fatal outcome. At any rate, it is certainly true that in most fatal cases, on examining the lung, one sees, not a sharply localized lesion, but an extending lesion frequently involving several lobes. This progressive extension seems to bear some relation to the virulence of the organism. With organisms of low virulence, the body is able to resist the infection, as regards both the spread of the local lesion and the general infection.

We have made quite extended studies to learn something of the nature of the general resistance of the body to the pneumococcus infection and its effects. It would seem that in pneumonia with its sudden crisis—one of the most startling and dramatic events with which the physician has to deal—an ideal opportunity would be offered to learn the nature of the process of recovery. It must be borne in mind, however, that only in certain cases does such a critical change in the patient occur. Of about 10,000 cases collected by Musser and Norris[23] the temperature fell by crisis in only about half. In the other cases it is difficult to determine with accuracy just when the change in the patient's condition occurs. It is therefore a mistake to think

23. Musser and Norris: In Osler's Modern Medicine, Philadelphia, 1907, ii, 537.

that in pneumonia we have a sudden change from susceptibility to resistance. More likely the process is a gradual one, and the marked change in the patient's condition occurs when the resisting factor, which increases gradually, reaches a degree sufficient to be effective. This factor of resistance may not be a single one, but the result may be due to a summation of several factors.

It has been suggested that the crisis represents a kind of anaphylactic reaction. It is known that following serum anaphylaxis there occurs a period during which the animal is in a refractory state. If the intoxication in pneumonia is due to peptone-like substances derived from the bacterial protein, it is possible that the crisis is a form of cumulative shock, following which the patient is refractory. Little is known, however, concerning such prolonged anaphylactic intoxication, and the nature of antianaphylaxis is still so obscure that it does not seem profitable to dwell longer on this theory, attractive as it is.

The fact that the crisis usually occurs in about seven days is strongly suggestive that the reaction is a true immunity reaction, since it is about in this time that antibodies appear in the blood in their maximum concentration, as we know from artificial immunization.

The view that recovery in pneumonia is due to the production of immune substances presupposes that at the end of an attack of pneumonia the patient is immune. We know from experience, however, that this is not so, or if immunity is present, it is of very short duration. I have seen a patient return to the hospital with a typical attack of pneumonia two days after discharge from a previous attack. Moreover, it is well known that a person may have repeated attacks; in fact, one attack seems to render a person more susceptible. It is quite possible, however, that the relative natural immunity of man requires only a very slight assistance in the shape of acquired humoral immunity in order to render the body able to overcome the infection, and following this the immune bodies may very quickly disappear from the blood. The attempts to demonstrate the appearance of known immune substances in the blood during and following an attack of pneumonia have not previously been very successful. An increase of the ordinary bactericidal substances which act in conjunction with complement has not been proved. Most observers have found that the pneumococci grow quite well in the blood-serum of patients recovering from pneumonia, even in the serum of immunized animals.

It has been asserted that by combining the leukocytes and serum of pneumonia patients, or by using the defibrinated blood, definite differences may be demonstrated between the blood of normal persons and that of patients during or following the crisis. None of these experiments seem to me free from objections. There does not seem to be

sufficient evidence for the conclusion that the recovery is due merely to an increase of opsonins, though Clough,[24] who has studied the phagocytic activity of the serum obtained after crisis or lysis in a series of eleven cases, found in six of these definite power of the serum to bring about phagocytosis of virulent pneumococci. In two of these cases the serum was tested before crisis and showed no such action. In his experiments, with one exception, the phagocytic activity was limited to the homologous strain. It has been stated by Rosenow that a difference exists as regards phagocytic activity between the leukocytes of patients with pneumonia and those of normal persons, though the results of others (Tunnicliff and Eggers) do not confirm these conclusions.

TABLE 1.—Protective Power of Serum of an Untreated Patient—

Quantity of Culture in c.c.	Quantity of Serum in c.c.	Virulence; no Serum	Control; Normal Serum	Three Days Before Crisis	Two Days Before Crisis
0.1	0.2	— *	—	—	24†
0.01	0.2	—	—	—	42†
0.001	0.2	27†	42†	42†	29†
0.0001	0.2	42†	42†	66†	42†
0.00001	0.2	50†	42†	32†	42†
0.000001	0.2	45†	43†	42†	47†

* Animals protected as shown by survival. † No. of hours before death of animal

Wolff[25] has attempted to show the increase of phagocytic power in the blood of pneumonia patients by making a composite curve combining the number of leukocytes with the opsonic index. We feel, however, that the errors in the usual opsonic technic are too great to justify his conclusions.

Of more importance are the experiments showing an increased protective power for mice of the blood of patients after recovery from pneumonia, as tested against known lethal doses of pneumococci. Neufeld has shown that while normal human serum had no protective action, that obtained from certain patients following the crisis had a definite effect. Strouse,[26] and Seligmann and Klopstock,[27] however,

24. Clough: Bull. Johns Hopkins Hosp., 1913, xxiv, 295.
25. Wolff: Jour. Infect. Dis., 1906, iii, 731.
26. Strouse: Jour. Exper. Med., 1911, xiv, 109.
27. Seligman and Klopstock: Ztschr. f. Immunitätsforsch., Orig., 1909-10. iv, 103.

failed to demonstrate such changes. Studies on this question were therefore undertaken by Dochez[28] on a series of cases. The method used was the following: Specimens of the patient's serum were obtained on various days both before and following the crisis. When possible, the organism against which the serum was to be tested was cultivated from the patient, either from the blood or sputum. In case the pneumococcus, when isolated, was of low pathogenicity, the virulence was raised by successive animal passages until a dose of 0.000001 c.c. of a broth-culture was sufficient to kill. Twenty-four-hour broth-cultures fresh from animals were used for infection, and the serum and varying quantities of the culture were mixed in the barrel of a

—WITH LOBAR PNEUMONIA AT VARYING STAGES DURING THE DISEASE

Serum Obtained						
One Day Before Crisis	Three Hours After Crisis	Two Days After Crisis	Four Days After Crisis	Five Days After Crisis	Seven Days After Crisis	Eight Days After Crisis
24†	32†	29†	42†	42†	32†	24†
20†	75†	42†	42†	27†	—	32†
42†	66†	*	*	*	42†	42†
66†	*	*	49†	42†	44†	42†
42†	*	*	*	42†	66†	45†
66†	*	*	*	*	45†	96†

injected.

syringe and immediately injected intraperitoneally. The appearance of protective substances in the blood could then be detected, as shown by the protocol from one such experiment (Table 1).

The serums from fourteen cases of pneumonia were so studied. In ten of these the serums were tested against homologous organisms. Of these ten cases all but one showed at some time the appearance of protective substances in the blood. Of the serums from four cases tested against stock cultures, only one showed any protective power. The amount of protection was never very high, though in some instances 0.2 c.c. of serum were able to protect against one thousand times the minimal lethal dose. The time of appearance of the protective substances varied somewhat, though in seven instances protective substances either appeared for the first time or showed a marked increase in amount at the time of crisis or, in case of lysis, during the

28. Dochez: Jour. Exper. Med., 1912, xvi, 665.

period when the symptoms were abating. In two cases the serum taken during the period of defervescence exhibited little or no power of protection, even against homologous strains, and it was not until some time later, in one case sixteen days, that the presence of protective substances in the blood was demonstrated.

Clough[24] later carried out a similar set of experiments, and in nine out of twelve cases the serums after crisis or lysis showed definite protective power against homologous strains. The technic used differed somewhat from that employed by Dochez and the results were not so striking, but show well that in most cases the serum acquires definite protective power.

These experiments are of great importance as showing, first, that in many cases, at least, protective substances appear in the blood of patients recovering from lobar pneumonia, and second, that these protective substances in many cases are active only against the race of organism concerned in the infection. These experiments, however, do not yet establish that crisis or recovery in pneumonia is due alone to the development of these protective substances in the blood. As already stated, in certain cases they cannot be demonstrated. It is altogether probable, however, that they play some part in the final outcome. As to the nature of the substances which are most active, it is impossible at the present time to state.

Probably recovery from pneumonia occurs when the growth of the organisms is inhibited and their toxic effects neutralized. It is impossible to state which comes first. It is conceivable that if the toxic effects of the bacteria are neutralized, the body is readily able to cause their destruction, since it is possible that pathogenicity depends entirely on toxicity. There is some evidence, as I shall show, that immune serums are antitoxic. With the present technic it has not been possible to demonstrate increase of antitoxic power of the patient's serum during the crisis.

In the immune-body theory of the crisis, the local lesion is left entirely out of consideration. It is quite evident that in pneumonia we are dealing, not merely with a septicemia, but with a condition in the lung which has a very important bearing on the termination. The involved portion of the lung forms a solid mass in which are growing numbers of micro-organisms. In each alveolus are fibrin, leukocytes, red blood-corpuscles and bacteria, and in the spaces free serum. Now it is known that as the process advances, the number of leukocytes becomes greater and greater. Resolution finally occurs almost certainly as a result of this increase and associated breaking down of the leukocytes, and with this the setting free of ferments which bring the fibrin into solution. The fact that this does not occur earlier is due to the overbalancing of the leukocytic ferments by the antiferments of the serum, and the lytic ferments become active only when the relation

between leukocytes and serum is in favor of the former. It is conceivable that recovery only ensues when such a balance occurs and when with the solution of the fibrin, tension is relieved and there is an outlet for the exudate. Instead of the surgeon inserting a knife, nature does the work by injecting a ferment.

It is quite probable, moreover, that during resolution other factors than the purely mechanical are at work. With the solution of the exudate, numerous substances are formed which have a direct destructive action on the bacteria. Such substances as the soaps of fatty acids, which are known to have such a destructive action, have been demonstrated in the resolving lung by Lamar.[29] Moreover, it is well known that during the growth of pneumococci outside the body, substances are formed in the culture medium which themselves are destructive. It is quite probable that such substances are being formed in the lung and they may aid in bringing about destruction of the pneumococci. Against the view that crisis depends mainly on resolution of the exudate, however, may be brought the very evident and conclusive objection that they do not necessarily occur synchronously. Resolution may be long delayed, or resolution may be occurring in one part of the lung while the process is advancing in another.

That leukocytes play some part in recovery is rendered probable by the experiments of Klein and Winternitz.[30] They have shown that when rabbits are treated with benzene, a leukotoxic substance, the animals rapidly succumb to pneumococcus infection, whereas when they are treated with toluene, which is a similar substance but which has no effect on the leukocytes, no decreased resistance is seen. Whether the chief function of the leukocytes consists in limiting the local infection, in which they undoubtedly play a rôle, or in aiding in the development of a general immunity is not indicated by these experiments. Clinicians, however, have long been of the opinion that a low leukocyte content of the blood is unfavorable.

It is not unlikely that in recovery all of the factors mentioned play a part. The destruction of the bacteria in the lung lesion may depend on local factors quite different from those responsible for the destruction of the bacteria in the circulating blood. From present knowledge it would appear that the growth of bacteria in the blood is the most serious part of the pneumonic process, and it seems that this, at least. is influenced by the appearance of circulating anti-bacterial substances.

METHODS OF CURE

It has been known since 1891 that susceptible animals may be rendered resistant to the action of pneumococci by the injection of increas-

29. Lamar: Jour. Exper. Med., 1911, xiii, 1.
30. Klein and Winternitz: Jour. Exper. Med., 1913, xviii, 50.

ing and properly spaced doses of pneumococci, beginning with the dead organisms. Moreover, it was early shown that if a very small amount of the serum of the immunized animal is injected into a second animal, this animal for a short time is also immune. These experiments are so striking and fundamental that it is no wonder that various attempts have been made to prepare and use such serums therapeutically. The clinical results, however, have not been convincing. Certain observations made principally by Neufeld and his collaborators and other observations made in our own laboratory, suggest reasons why such results have not been satisfactory and methods for overcoming the difficulties.

Opinions have differed as to whether or not an immune serum produced by the injection of a given race of pneumococci into an animal is effective against all races of pneumococci. The first accurate studies on this problem were made by Neufeld and Händel.[31] They tested a so-called univalent serum against various races of pneumococci. While this univalent serum was protective in mouse experiments against fifteen strains studied by them, against other strains the serum had practically no effect. They decided that these atypical strains were not *Serum-fest* in the ordinary sense of the term, since the serum obtained during convalescence from one of the patients, from whom one of these organisms had been isolated, protected mice against the homologous strain and also against one of the other atypical strains, but did not protect against the typical strain. They then produced an immune serum against one of the atypical strains to see whether all atypical strains could be affected by this immune serum, but found this not to be true. In their further studies they found that the second immune serum, which they called *Serum Franz* protected against only three of the atypical strains isolated by them, but failed to protect against three other strains. These latter three strains they further showed to be individual in their reactions. Neufeld and Händel did not have access to a large number of patients with pneumonia from whom to obtain cultures, and could not determine the frequency of occurrence of atypical types, nor could they make extended studies on grouping of the organisms on a biological basis, though from their studies the possibility of making such a grouping was most evident.

With the opening of the Hospital of the Rockefeller Institute in October, 1910, patients suffering from lobar pneumonia were admitted for treatment and study, and an extended study was commenced of the pneumococci obtained in these cases. An immune serum was pre-

31. Neufeld and Händel: Ztschr. f. Immunitatsforsch., 1909, iii, 159; Arb. a. d. k. Gsndhtsamte, 1910, xxxiv, 169; ibid., 1910, xxxiv, 293; Berl. klin. Wchnschr., 1912, xlix, 680.

pared by injecting a horse with a culture of pneumococcus obtained from Professor Neufeld, the same race he had employed in the production of his immune serum. The protective power of this serum for mice was tested against a number of races of pneumococci cultivated from a series of cases of pneumonia. A report by Dochez[33] gave a preliminary report of this study, indicating that this serum protected against only about half the races studied. It was therefore evident that if such a serum were employed therapeutically, an effect could be expected at the most in only about half of the cases treated.

Experiments[34] were then undertaken to determine whether it would be possible to make a biologic classification of pneumococci obtained from cases of pneumonia, based on their reaction to different serums in protection experiments. Rabbits were therefore immunized to each of the races which were not acted on by the horse-serum, which we have called Serum 1, and the protection afforded by these different rabbit serums against all the other races tested. A considerable number were found to show cross-protection, that is, a serum prepared by injection of one of the number acted on all the races of this group. A horse was then immunized to one of this group and the serum is called Serum 2. In this way it has been possible to divide the pneumococci obtained from cases of pneumonia into four groups. In Group 1 are included all the races against which Serum 1 is effective. In Group 2 are included all those against which Serum 2 is effective. Whether the races included in this group correspond with the organisms described by Neufeld as acted on by his immune serum *Franz* is not known at present. In Group 3 are placed all the organisms of the so-called *Pneumococcus mucosus* type. These organisms have very large capsules and produce a sticky exudate in animals. In Group 4 are included all the races against which Serums 1 and 2 are not effective and which, from their other properties, do not belong in Group 3. Animals may readily be immunized to any member of this Group 4, and the serum of the immunized animal is protective against the race used for immunization. In no instance, however, has this serum been found effective against any other race of this group or against the organisms of the other groups. So far as cultural and morphologic characters are concerned, no constant group differences have been discovered between the members of Groups 1, 2 and 4. By means of the agglutination reaction, however, it has been found possible to group them in exactly the same manner as by protection experiments.

33. Dochez: Jour. Exper. Med., 1912, xvi, 680.

34. Dochez, A. R., and Gillespie, L. J.: A Biologic Classification of Pneumococci by Means of Immunity Reactions, Jour. Am. Med. Assn., 1913, lxi, 727.

As previously stated, the members of Group 3 differ from the others somewhat in their morphologic and pathogenic characters. They differ further in the fact that while animals may be very highly immunized to them, the serum of such animals possesses no protective power; they induce active but no passive immunity. Studies have been undertaken by Hanes to learn on what factor this failure to produce passive immunity depends. It was found that the serum of the immunized animals not only does not protect, but also has no agglutinating power. It has been known that certain encapsulated bacilli also fail to be agglutinated by immune serum. Porges,[35], [36] however, has shown that such bacilli are agglutinated by the serum of immunized animals, provided the bacilli are previously treated so as to destroy their capsules. This method was therefore employed by Hanes[37] in studying these cocci. Six typical races of *P. mucosus* obtained from

TABLE 2.—AGGLUTINATION OF PNEUMOCOCCUS MUCOSUS

Organism No.	Immune Serums Nos.								Normal Rabbit Serum
	19	26	42	54	68	96	I	II	
19	+	+	+	+	+	+	—	—	—
26	+	+	+	+	+	+	—	—	—
42	+	+	+	+	+	+	—	—	—
54	+	+	+	+	+	+	—	—	—
68	+	+	+	+	+	+	—	—	—
96	+	+	+	+	+	+	—	—	—
I	—	—	—	—	—	—	+	—	—
II	—	—	—	—	—	—	—	+	—

cases of pneumonia, were studied. The bacteria were treated with dilute hydrochloric acid and heated for fifty minutes at 80 C. (176 F.). The fluid was then neutralized and the bacteria so treated tested for agglutination. Controls were made with members of Groups 1 and 2 treated in the same way. The results were definite and striking. Agglutination of all the six races of *P. mucosus* occurred promptly with all six immune serums obtained by inoculating each of a series of rabbits with one of these races. No agglutination of Pneumococcus 1 or 2 occurred with any of these serums, and *P. mucosus* was not agglutinated by either Serum 1 or 2. (See Table 2).

These experiments show that, so far as tested, all the organisms of the *P. mucosus* type belong in one biological group differing from

35. Porges: Wien. klin. Wchnschr., 1905, xviii, 691.
36. Porges and von Eisler: Centralbl. f. Bakteriol., First Abt., Orig., 1906, xlii, 660.
37. Hanes: Jour. Exper. Med., 1914, xix, 38.

those of the other groups. In order to show the relation of these organisms to streptococci, the method of complement-fixation was employed. With this method there occurred a considerable amount of cross-fixation among the various races of pneumococci, including *P. mucosus,* but no cross-fixation was observed in testing the complement-fixing powers of *Streptococcus mucosus* or *S. pyogenes* serums. It therefore seems evident that *P. mucosus* is really a variety of pneumococcus, and that biologically it forms a distinct variety of this organism.

Further studies of the various members of the *P. mucosus* group, to see if any were affected by the immune serum *in vivo,* were all negative. None of the serums were able to protect mice, even against the homologous organism. These experiments and also observations of Gruber and Löhlein seem to indicate that the failure of such serums

TABLE 3.—Classification by Protection and Agglutination

	Number	Per Cent.
Group 1	35	47
Group 2	13	18
Group 3 (P. mucosus)	10	13
Total typical	58	78
Group 4 (heterogeneous)	16	22
Total heterogeneous	16	22
Total number	74	

to protect is in some way related to the formation of the thick, mucoid capsules by these organisms. As soon as the bacteria commence to grow in the body, capsules are formed which prevent the action of the immune serum. By the methods employed by Dochez and Hanes, it has been possible to study the races of pneumococci obtained from a series of cases of pneumonia. The classification by protection and agglutination experiments of sixty-two organisms so obtained gave results as shown in Table 3. In every instance in which an organism could be placed by protection in one of the groups described, the agglutination reaction has corresponded.

The races placed in Group 4 have been called heterogeneous, since each race, so far as studied, appears independent, and no grouping of the members on a biologic basis, by means of protection or agglutination, is at present possible. Table 4 shows the results of the study of agglutination with these races.

Gillespie[38] has also made a study of the various races, using the method of agglutination of bacteria by acid, as introduced by Michaelis. The results also show certain group differences in the agglutination of the various races.

Recent observations by Rosenow indicate that by certain methods it is possible completely to change the characters of the organisms of the entire streptococcus-pneumococcus group, so that one may be transformed into the other, even *S. hemolyticus* into a typical pneumococcus and vice versa. It has long been thought that the various closely related bacteria must originally have had a common source and have become differentiated by processes of adaptation. It is remarkable, however, that the changes can occur in such a short period of time as shown by Rosenow, even though they are subjected

TABLE 4.—Strains of Heterogeneous Group Tested and Results of Agglutination

Serums Pneumococcus	Pneumococcus Strain Numbers													
	1	2	34	36	37	38	52	55	60	62	71	76	82	102
1	+	0	0	0	±	0	0	0	0	0	0	0	0	0
2	0	+	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	+	0	0	0	0	0	0	0	0	—	0
37	0	0	0	0	+	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	+	0	0	0	—	0
71	0	0	0	0	0	0	0	0	0	0	+	0	—	0
76	0	0	0	0	0	0	0	0	0	0	0	+	0	0
82	0	0	0	0	0	0	±	0	0	0	0	0	+	0
Polyvalent, 34, 38, 62.	0	0	0	0	0	+	0	+	0	+	0	0	—	0

to extreme changes in environment, as has apparently been done. Some experiments performed in my laboratory several years ago by Strouse indicated that sudden mutations might appear in this group. Important as these experiments are, they do not have an immediate bearing on the pneumonia problem, except as regards the origin of the infection.

As regards the course of the disease and the possibility of specific methods of cure, the possibility of transformation of type of the organism concerned is not significant. In all the studies of organisms obtained at different times from the same case, in no instance have there been any indications of a change in type; the type first isolated has always subsequently been found. Moreover, many of these strains have now been cultivated for a long time outside the body, both in artificial mediums and in repeated passages through animals, some of

38. Gillespie: Jour. Exper. Med., 1914, xix, 28.

them for several years, and they have in all cases retained their original characteristics. The results of the present year are not included in Table 3. This year the largest number of cases has been due to organisms of Type 2. It is possible that the prevalence of cases due to the various types varies from year to year and in different places, which may explain the variation in mortality observed in different times and places. The mortality of our limited number of cases due to organisms of different types is shown in Table 5. The most striking fact is the low mortality due to organisms of Type 4. Further observations may possibly change our ideas with regard to the relative severity of cases due to organisms of the different types.

In addition to the fact that there are immunological differences in the pneumococci concerned in pneumonia, there is probably another reason why the use of immune serum has not proved efficacious in the past. The method of employment of such immune serum has been to

TABLE 5.—MORTALITY IN PATIENTS INFECTED WITH VARYING TYPES OF ORGANISMS

Infection Type	No. Patients	Patients Died	Per Cent.
1	34	8	24
2	13	8	61
3	10	6	60
4	15	1	7
Total.....	72	23	32

use small doses, from 10 to 20 c.c., usually given subcutaneously. Neufeld and his assistants, by titrating immune serum against varying doses of pneumococci and making injections into mice, have concluded that in order to obtain protective power a certain proportion of serum in relation to body-weight is required. This concentration they have called the *Schwellenwert* or threshold concentration, and from experiments on mice they estimate that for man the dose of serum employed by them must be at least 77 c.c. Undoubtedly, however, this *Schwellenwert* must vary enormously under different conditions, depending on the virulence of the organism, the time the serum is given, etc. In any case their experiments indicate that the serum must be given in very much larger amounts than has hitherto been done, in order to obtain curative results. They concluded that such an antibacterial serum does not obey the law of multiple proportions. This is undoubtedly true for the conditions employed by them, namely, injections made separately in different parts of the body, and is also true for the conditions present

in the therapeutic injections in man. Dochez has shown, however, that when the serum and cultures are mixed before injection, such a serum does obey the law of multiple proportions up to a certain point, but there is a maximum degree of infection against which no amount of serum, however large, is able to protect. It would therefore seem that one of the factors of the protective mechanism must be supplied by the body, and that, when the infection is very great, a sufficient number of immune bodies may be supplied by the administration of serum, but the body cannot react to a sufficient extent adequately to supply this second factor. This suggests that in order to obtain results from serum, it should be administered early, before the infection has reached too extreme a grade, beyond which no amount of serum can be effective, and also offers a possible explanation of the fact that in certain cases, such as the one which I shall mention, case 4, the serum seems to have absolutely no effect. The nature of this additional factor is not known. If, as previously stated, the serum owes much of its effect to its bacteriotropic power, the number and activity of the leukocytes may be the additional factor. That this factor may be stimulated is shown by the results of artificial immunization, whereby very much larger doses of bacteria are resisted than can be protected against by immune serum.

These experiments have indicated that for the successful employment of immune serum in pneumonia, it must be employed fairly early and in large doses, and a serum must be used which is effective against the variety of organism causing the infection. We have been able to produce a serum of very great efficiency against organisms of Type 1, and one effective against organisms of Type 2. So far it has been impossible to produce a serum effective against *P. mucosus,* and, for the reasons stated, it would only be practical to treat cases of pneumonia due to organisms of Type 4 with homologous serums. This is of less importance, however, since the cases due to these organisms are apparently of mild grade.

To employ the serum effectively in cases due to organisms of Types 1 and 2, it has been necessary to devise a method for quickly determining in each case the type of organism concerned. This has been done and the following method is employed. When a patient with pneumonia is admitted to the hospital, a culture is immediately made from the blood and also one from a portion of sputum coughed up from the lung, or, when this is not obtainable, a culture is made directly from the lung by the insertion of a needle. This procedure seems to be without danger. When there are large numbers of organisms in the sputum, a culture may be obtained most rapidly by injecting the washed sputum into the abdominal cavity of a mouse. After four or

five hours the peritoneal cavity may be washed out with salt solution and the cells thrown down in the centrifuge; a suspension of the organisms is thus obtained. In whatever way the culture is obtained, the agglutination test is at once applied. If the organism fails to agglutinate with either Serum 1 or Serum 2, it is, of course, useless to undertake serum treatment. If, however, one of the serums agglutinates the organism, treatment may be commenced at once with the appropriate one.

So far it has been possible to treat only a comparatively small number of cases. Twenty-three cases have been treated with the serum. Of these, fifteen were due to organisms of Type 1 and eight

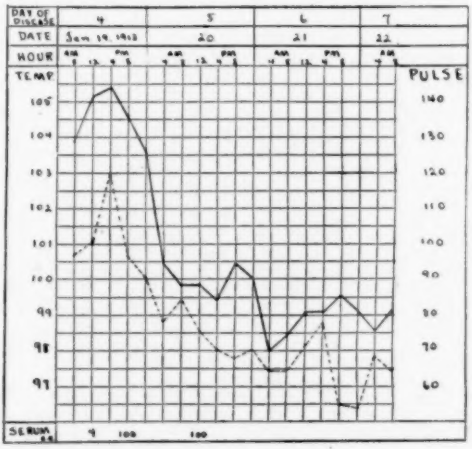

Fig. 1.—Case 1, E. S., No. 988, aged 20 years, admitted January 18, 1913, on the third day of the disease. Physical signs showed involvement of the lower left lobe and also signs of beginning involvement in the lower right lobe. Blood culture was negative. Pneumococci obtained from sputum were of Type 1. The patient was quite ill on admission, respirations were labored and there was cyanosis. His condition was markedly improved on the day following the first administration of the serum, as shown by the temperature and pulse curves. Recovery was uneventful except that slight signs in the lungs persisted for several weeks.

to organisms of Type 2. The method of administration of the serum was the following: When admitted, the patient was given 0.5 c.c. of serum subcutaneously to discover if hypersensitiveness existed. As soon as the type of organism was determined, from 50 to 100 c.c. of the serum, diluted one-half with salt solution, were injected intravenously. The condition of the patient served as a guide in the later

treatment. Usually the serum was not administered oftener than every twelve hours. The patients treated received totals of from 190 to 460 c.c. of serum, except one, who received a total of 700 c.c. of serum. The patients treated were all seriously ill. They were treated in series. Every case infected with a pneumococcus of Type 1 or Type 2 was treated. Of the fifteen cases due to Pneumococcus 1, all of the patients recovered but one, and of the eight cases due to Type 2, two patients died. One of these patients objected to the treatment and would not allow its continuation, so it was not thoroughly carried out. When we consider that the mortality among the untreated patients infected with Types 1 and 2 is very high (Table 5), the result is certainly not discouraging. It must also be remembered that so far most of our cases have been admitted late in the disease. Treatment was commenced on the third day in six cases, on the fourth day in five cases, the fifth day

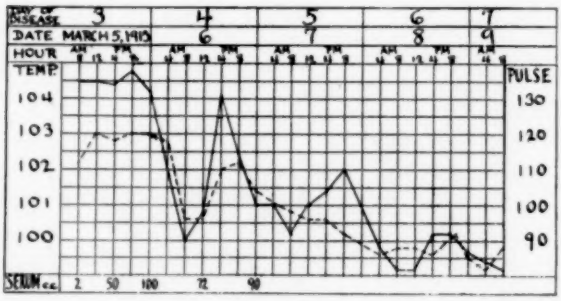

Fig. 2.—Case 2, B. G., No. 1175, aged 36 years, admitted March 4, 1913, on the second day of the disease. There was slight involvement at the base of the right lobe. Blood culture was positive. Agglutination test showed Type 1 organism. Treated with serum on day following admission. The signs of involvement in the right lower lobe became more distinct, but there was no apparent extension of the involvement beyond this lobe during the course of the disease. Following injection of the serum the patient's general condition improved. The patient complained of some urticaria and joint pains beginning on the twelfth day.

in six cases and the sixth day in six cases. If treatment can be commenced early, it is probable that the results will be even better than they now are. It is to be hoped that during this winter a large number of cases may be treated early in the disease. Effective treatment in the cases due to Types 1 and 2 should cut down the total mortality due to pneumonia very materially, as it has already done in our hands. I prefer at present, however, not to lay the main stress on the mortality statistics, since these are not large enough to be conclusive, but to refer to other criteria which we possess as to the efficacy of the serum.

Let us first consider the effect on the clinical course of the disease. Following practically all the injections, a reaction has occurred. The temperature usually rises and then falls, but does not necessarily remain low. In two instances the rise of temperature has been marked. In the other cases the rise of temperature following the injection was only a degree or so. In all the cases except the fatal ones, the serum has apparently had an ultimate favorable effect in lowering the temperature and shortening the course of the disease, though, of course, this is a very difficult matter of which to be absolutely sure. In no case was one injection of the serum sufficient to bring on a crisis.

Figures 1, 2, 3 and 4 show the effect of treatment on the temperature curves in certain of the cases. It is manifest that wrong impressions may be produced by the exhibition of temperature curves unless all the curves of a series are given. To avoid this difficulty, so far as

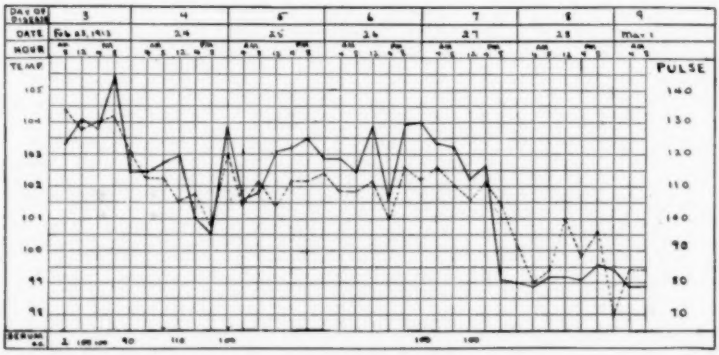

Fig. 3.—Case 3, M. L., No. 1091, aged 30 years, admitted February 22, 1913, on the second day of the disease. Blood culture showed a growth of pneumococcus, Type 1. Physical signs indicated involvement of the right lower lobe with slight involvement of the left lower lobe. The patient's condition improved following the treatment and the signs in the lungs became less well marked. On the sixth day, however, the patient's condition was again worse, the temperature was higher and the pulse more rapid, so that treatment was commenced again, following which the patient's condition markedly improved and he made a rapid recovery except for symptoms due probably to serum sickness on the twentieth to the twenty-fifth days of the disease.

possible, however, a curve from each group of cases is shown. Figure 1 represents the curve of a case in which the serum apparently had a marked effect, the temperature falling promptly and in a striking manner. Figure 2 indicates a temperature curve in a case in which the temperature fell following the administration of serum, but several doses were necessary before the temperature remained low. Figure 3 shows the temperature curve in a case in which there were apparent

effects of the administration of serum, but after the administration of serum was discontinued, the temperature curve rose and only fell after further large doses of serum were administered. In Figure 4 is given a temperature curve in which the serum had apparently no effect and the patient died on the seventh day.

All the patients seemed to feel better following the injection of the serum, and in a number of cases the apparent lessening in the degree of intoxication was very manifest. When the treatment was commenced early, no extension of the involvement of the lung occurred. On the other hand, there was no special tendency in the treated cases for the lung lesion to resolve rapidly. If anything, there seemed to be a tendency for resolution to be delayed in these cases. This has been noted by others in certain cases treated by serum.

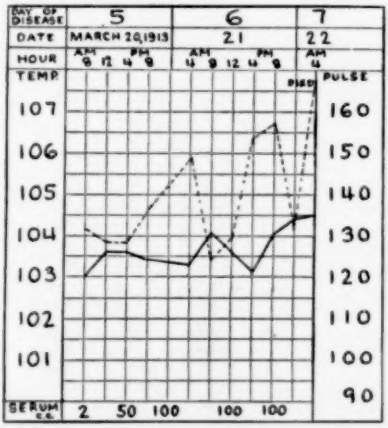

Fig. 4.—Case 4, F. W., No. 1090, aged 36 years, admittted March 19, 1913, on the fourth day of the disease. The patient was extremely ill on admission. There was involvement of the right upper and lower lobes. Blood culture and culture from the lung showed the presence of pneumococcus, Type 1. There was some irregularity of the heart. Following treatment on the fifth day, the patient seemed better. The blood culture was negative. The pulse continued rapid and showed an extrasystolic irregularity, however, and the patient showed a very marked cyanosis. In spite of the active serum treatment, the patient died on the seventh day.

More important than the foregoing criteria, however, as indicating an effect of the serum, are the following observations, since they have depended solely on objective procedures: First to be mentioned is the effect of the serum on the organisms in the blood. In ten cases pneumococci were isolated from the blood before the treatment was commenced. In all cases blood-cultures were made before each treat-

ment; and in all of these ten cases after one treatment and before the second (or within from eight to twelve hours) the blood had become sterile. The conclusion seems justified, therefore, that one large dose of active serum given intravenously is sufficient to sterilize the blood. It also seems certain that if the organisms are not present in the blood, the administration of the serum will prevent their invasion. Second, in a previous study of the protective substances in the blood-serum of patients with pneumonia, it has been shown that, as a rule, the appearance of protective substances in the blood, when demonstrable, coincides rather sharply with the period of critical fall in temperature and the disappearance of symptoms. Before the crisis they are not present in the blood in any measurable degree.

TABLE 6.—Protective Power of Serum B. G.; Treatment Commenced on the Third Day

Quantity of Culture in c.c.	Virulence; No Serum	Quantity of Serum in c.c.	Serum Obtained			
			Third Day, Before Treatment	Third Day, 6½ Hours After Treatment	Fourth Day, After Treatment	Ninth Day, Five Days After Last Treatment
0.001	—	0.2	16†	*	*	† Days
0.0001	—	0.2	20†	† 5 Days	*	*
0.00001	24†	0.2	24†	*	*	*
0.000001	28†	0.2	24†	*	*	*

* Animal protected as shown by survival. † Time in hours before death of animal injected.

A similar study has been made by Dochez of the protective substances in the serum in a number of the cases of pneumonia treated with serum. In all the cases studied, it has been possible to demonstrate the appearance of such substances in considerable amounts in the serum very shortly after the administration of one dose of the immune serum, even when this serum has been administered early in the disease, at a period when such protective substances are otherwise never present. (Table 6). These substances persist, and in case they play a part in the mechanism of recovery, as was concluded from the previous study, it is evident that their appearance indicates a favorable action of the immune serum.

The results obtained, therefore, from the clinical and laboratory study of this series of cases of pneumonia treated by the injection of large amounts of appropriate serum, seem to indicate that a method has been devised for the successful specific treatment of at least a portion of the cases of acute lobar pneumonia. Studies on the treatment

of pneumonia by the intravenous injection of the Neufeld-Händel immune serum have been made by Beltz,[39] Weitz[40] and Geronne.[41] In none of these series, however, were studies made of the type of organisms concerned in the infection of the cases treated, and in all of the cases the amount of serum administered was too small, judging from our own experience, to be of value.

The mode of action of the immune serums is still somewhat obscure. It is quite evident that there is an antibacterial action, inasmuch as the bacterial invasion of the blood is prevented. The action on the local lesion, however, is less evident. It is probable that here the organisms can less readily be reached by the serum, though apparently in most cases the growth of the bacteria in the margins of the lesion has been inhibited, as shown by prevention of spread of the process. In addition to the antibacterial action, the clinical cases show a definite change as regards intoxication. It is possible, of course, that this is entirely associated with the destruction of the organisms. Certain experimental work, however, has indicated that the serum may possess some antitoxic effect.

When the immune horse-serum is added to the toxin obtained by dissolving pneumococci in bile, it is found that such a serum has a well-marked effect in inhibiting the hemolysis of sheep corpuscles by this toxin. When it is added to the toxin in doses of 1 c.c. of serum to 4 c.c. of toxin and placed at 37 C. (98.6 F.) for one-half hour, the effect of the toxin when injected into guinea-pigs is diminished or entirely prevented. These effects of the immune horse-serum are much less specific, however, than are the protective or antibacterial effects, since the Serum 1 acts on both Toxins 1 and 2, though most markedly on Toxin 1. Serum 2 also shows a similar diminution in specificity in antitoxic action as compared with antibacterial action. These experiments offer some evidence that part of the effect of the immune horse-serum is antitoxic, admitting, of course, that the toxic substances obtained from the bacterial bodies are responsible for the intoxication.

An effort has been made to obtain a pure antitoxic serum by the injection of the toxin alone into animals. Rabbits have been immunized by the repeated injection of this toxin and a sheep also has been highly immunized. The sheep-serum and also the immune rabbit-serums show antitoxic power, as indicated by anti-hemolytic action and also by neutralizing effect on the toxin, as tested by injection into guinea-pigs. The effects, however, are less marked than those of

39. Beltz: Deutsch. med. Wchnschr., 1912, xxxviii, 14.
40. Weitz: Med. Klinik, 1912, viii, 1072.
41. Geronne: Berl. klin. Wchnschr., 1912, xlix, 1699.

the antibacterial horse-serum. These antitoxic serums are also protective against the living organisms, as shown by tests on mice. The protection, however, while fairly high, is less well marked than that of the horse-serums. The protection is not so specific as that of the horse-serum, since the serum produced by the injection of toxin prepared from an organism of Type 1 is not only protective against this organism, but also, though to a less extent, is protective against organisms of Type 2.

The interpretation of these experiments is attended with much difficulty. It is possible that these antitoxic serums may show protective power only because living organisms were introduced, since in the preparation of the toxin one cannot be positive that all organisms have been destroyed. These antitoxic serums, however, possess no power to cause agglutination, and this fact, together with their lessened specificity, suggests that we are dealing with serums which owe their power to other properties than those of the antibacterial serums. The experiments are of importance, moreover, since they indicate that immunity may be obtained against the substances contained in the bile extracts, and since the essential criterion of a toxin in the Ehrlich sense is that immunity may be obtained to it. Much more work will have to be done before such antitoxic serums should be employed therapeutically.

It is probable that in the future it will be possible to obtain the same therapeutic effects by the injection of much smaller amounts of serum than are now employed. Work now being carried on by Avery shows that the immune substances are all contained in the globulin fraction of the serum, and methods are now being devised for the concentration of the serum, so as to avoid the injection of a very large part of the serum protein which contains no immune substances. In this way it will probably be possible to avoid serum sickness, which has occurred in a number of our patients in from ten to ewelve days after the administration of the large amounts of horse-serum. This serum-sickness, while causing some discomfort to the patients, is not of any serious import, so far as we know.

It may be possible later to produce polyvalent serums that are efficacious. At present, however, and until the value of the special serum in the cases due to organisms of Groups 1 and 2 is unquestionably determined, it does not seem to be advisable to make such attempts. The objection is frequently raised that this method of treatment is very complicated. One may reply to this that so is the treatment of appendicitis.

At the present time I can do no more than mention the efforts along other lines that have been made to produce curative results by

specific measures. Most important studies were made by the late Professor Hiss in the treatment of bacterial inflictions by means of leukocytic extracts. So far as concerns pneumonia, the results of experiments on animals are not very convincing, but the brief clinical report of cases of pneumonia treated, as stated in the article published since his death, seems extremely favorable and promising. It is to be hoped that study along this line will be continued.

Lamar has devised a method for the local treatment of pneumococcus infections. He has shown that immune serum has a much greater effect on pneumococci treated with sodium oleate solutions than on cocci simply washed in salt solution. This action of the soap, however, is inhibited if the serum be added first or mixed with the soap solution before treating the bacteria. The inhibiting action of serum, however, may be prevented by the addition of small amounts of boric acid, as Liebermann and von Fennyvessy have shown. By combining the soap, serum and boric acid in proper concentrations, Lamar has found a mixture that is much more efficacious in the local treatment of experimental pneumococcus infections than is serum alone. The treatment of local infections, as meningitis, with such a mixture, using serum effective against the race of organisms concerned, should be tried in all suitable cases. It is doubtful, however, whether such a mixture can be employed intravenously.

A final possible method which may be rendered practical in the treatment of pneumonia is along the lines of chemotherapy, as laid down by Ehrlich. It has been generally held that such a method of treatment may be of value in protozoan infections, but not in diseases due to bacteria. Morgenroth[42] and his coworkers, however, have shown that a derivative of quinin—ethylhydrocuprein—has a specific action on pneumococcus infections in mice, and Wright has shown that this drug is bactericidal for pneumococci in the test-tube. The drug has been employed clinically, but cases of amblyopia developing have indicated that the toxic dose in man too closely approaches the curative dose to permit the safe administration of the drug. It is possible, however, that with further study, its toxic properties may be reduced without lessening its curative effect.

CONCLUSIONS

Much obscurity still exists concerning the mode of natural infection in pneumonia, though by animal experimentation many facts in regard to it have been discovered.

The symptoms in pneumonia are probably due to toxic substances derived from the bacterial cells.

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41. Morgenroth: Berl. klin. Wchnschr., 1911, No. 44.

The outcome is dependent on the virulence of the organisms concerned and on the ability of the body, first to limit the local infection, and second, to prevent the invasion of the blood by the organisms, as on the latter the outcome of the disease mainly depends.

Leukocytes probably play a part in the resistance, certainly as regards the local spread and probably also to some extent as regards the general infection.

The most important part in prevention of the general infection is probably played by immune substances contained in the serum. Such substances are present in the serum of immunized animals.

Pneumococci differ in regard to their immunological reactions and on these they may be divided into several groups.

In order to use immune serum effectively in treatment, as in prevention, it is necessary to employ the serum effective against the group of organisms to which the special organism causing the infection belongs.

Immune serums effective against two of the most important groups have been produced. This treatment has been carried out in a limited number of patients with promising results.

It is probable that the methods of application of such serums will be improved, and it is possible that the method may be combined with other measures directed toward other factors which are important for the outcome. In any case, facts regarding the nature of the disease are being disclosed, and the outlook, at least for lessening the ravages of this dreadful disease, is encouraging.

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THE COLOR-INDEX OF THE RED BLOOD-CORPUSCLES *

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At first sight it may seem strange that it should be necessary at this date to call attention to some of the earliest observations in clinical hematology. Nevertheless, it is not difficult to show that then, and even now, the principles underlying these observations have not always been kept clearly in mind.

It will be remembered that Hayem[1] called attention to the disproportionately high colorimetric values of the blood in pernicious anemia as compared with the colorimetric power of the same number of normal red blood-corpuscles. Quincke[2] noted an apparent increase in the average hemoglobin content of the red blood-corpuscles in pernicious anemia. Later Laache[3] made a systematic investigation of the hemoglobin content and of the number of erythrocytes of the blood in health and in disease, and defined precisely what we now call the color-index of the red blood-corpuscles. Laache obtained his values for the color-index by taking thirty men and thirty women in apparent good health and establishing for each set the average hemoglobin content and the average number of erythrocytes. The ratio of the averages, separately for the men and the women, was taken as 1. From this were derived all of the individual indices of that series and of all subsequent determinations with the same hemoglobinometer and in terms of the same counting apparatus. Determined in this way, the color-index becomes independent of any constant methodical error in either determination, provided that the dilutions and concentrations are really proportional to the scales of the measuring device employed.[4]

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* From the Second Medical Clinic of the University in Munich, Prof. Friedr. Müller, director.
* Aided by a grant from the Rockefeller Institute for Medical Research, New York.

1. Hayem: Du Sang, Paris, 1889. (Reference to first observations).
2. Quincke: Deutsch. Arch. f. klin. Med., 1880, xxv, 577.
3. Laache: Die Anämie, Christiania, 1883.
4. This was tested by Laache for the Malassez hemoglobinometer. Although he used different methods for enumerating the red blood-corpuscles, he reduced all of his figures to the terms of one method, having previously determined the differences between the several methods. From the apparent differences he concludes that his results on the enumeration of the red blood-corpuscles were only relative.

For the color-index determined by Laache, the extreme variations for normal blood were from 0.9 to 1.1. In pernicious anemia the index reached in one case 2.3, while in chlorosis it was sometimes as low as 0.4. After this work of Laache, the value of the high color-index as a diagnostic sign of pernicious anemia became generally accepted. It is extremely rare, if ever, that a high color-index determined according to the principles of Laache has been found in other diseases. Erich Meyer and Albert Heineke[5] observed a high color-index in normal fetal blood at 5 and 7 months. This observation was important for the interpretation of the blood-picture and histologic findings in pernicious anemia. W. Türk[6] was inclined to regard the results of Meyer and Heineke as being due to a methodical error in the hemoglobin determination. In their reply to Türk, Meyer and Heineke[7] showed that they followed the principle of Laache, which frees their results from any constant error of the methods.

In spite of Laache's work, the question of the color-index has arisen now and again, chiefly because most of the clinical methods of hemoglobin determination are purely relative and many of them highly unsatisfactory. It seemed desirable, therefore, to go over this field again with exact methods, and establish the absolute values for the total hemoglobin concentration and the number of erythrocytes of the blood in health and in disease. As our real aim was not only to establish beyond any remaining doubt the existence of a high color-index in pernicious anemia, but also to ascertain the nature of the phenomenon, it was first necessary to study separately the hemoglobin of anemic blood, particularly with reference to its iron content, oxygen content and light absorption. In 1908-1909 it was established in Tübingen that the light absorption, oxygen content, and iron content of the oxyhemoglobin of the blood in pernicious anemia, chlorosis and polycythemia was identical with that of the oxyhemoglobin in normal human blood.[8] This proved the constant composition of oxyhemoglobin in health and in the essential blood-diseases, at least as far as the significant factors, spectrum (color), oxygen absorption (function), iron content and molecular weight were concerned. After this had been established the question of the absolute values for the hemoglobin content and number of erythrocytes of the blood in health and disease was taken up in Munich in 1909. The results of these determinations, as well as the values for the color-index calculated therefrom, are given in the following pages.

5. Meyer, Erich, and Heineke, A.: München. med. Wchnschr., 1906, liii, 793.
6. Türk, W.: München. med. Wchnschr., 1907, liv, 220.
7. Meyer, E., and Heineke, A.: München. med. Wchnschr., 1907, liv, 327.
8. Butterfield: Ztschr. f. physiol. Chem., 1909, lxii, 173.

METHODS

The blood was taken in all cases from a vein of the arm, immediately defibrinated, and strained through gauze. Before each further step, the enumeration of erythrocytes, hemoglobin determinations, etc., the defibrinated blood was thoroughly shaken and continuously stirred while the pipettes were being filled.

Enumeration of Erythrocytes.—A Thoma-Leitz counting-chamber and pipette were used for all counts. The depth of the counting-chamber was determined micrometrically with the microscope, first with air, then with liquids of different refractive indices between the cover-glass and counting surface. The average of forty-five measurements of the distance between the under surface of the cover glass and the surface of the counting-chamber was 0.10 mm. ± 0.002 mm., provided interference bands of the third order were present throughout the film of water between the cover-glass and underlying glass plate. No counts were made unless these interference bands were present on all sides of the preparation. The total volume of the pipette was determined by weighing it empty and then filled with water. The volume of the capillary portion at the marks 0.5 and 1.0 was determined by weighing out with mercury. Each calibration was made in duplicate. The dilution, using 0.5 of the capillary content, was 186 instead of 200, and on using 1.0 of the capillary content, it was 101 instead of 100. Hayem's solution was used to prepare the dilutions for the counts.

Spectrophotometric Determination of Hemoglobin.—For the determination of the oxyhemoglobin content of the blood, the spectrophotometer of König, Martens and Grünbaum[9] was used with a white light (Nernst lamp) as a source of illumination. The spectrophotometric method is based on an empirical law, the so-called law of Lambert and Beer. When a bundle of parallel rays of monochromatic light enters under rectangular incidence and traverses a plane-parallel layer of a transparent homogeneous substance, the intensity of the bundle undergoes a certain diminution, apart from surface reflection. The amount of diminution depends on the nature of the substance, the wave-length of the monochromatic light and the concentration and linear thickness of the absorbing layer. The law of Lambert and Beer expresses the mathematical relation existing between the initial and final intensities of the monochromatic light in the solution and the concentration and linear thickness of the absorbing layer of the solution. The usual form is:

$$I' = Ie^{-klc}$$

in which I = the initial intensity, I' = the final (emergent) intensity in the solution, k = a constant which depends on the substance and on the wave-length of the light, l = the linear thickness of the absorbing layer, and c = the concentration of the solution. $\frac{I}{I'}$ is the quantity which is capable of measurement with the spectrophotometer. If k has been previously determined for the pure substance and l is known, then it is obvious that the concentration of the solution c, can be readily calculated when $\frac{I}{I'}$ has been measured. As a matter of fact k was determined with solutions of oxyhemoglobin from ox blood, after it had been previously shown in Tübingen with the Hüfner spectrophotometer that the optical constants of oxyhemoglobin from ox-blood and from human blood were practically identical in two regions in the spectrum.[10] The linear thickness of the absorbing layer was in all cases 2 cm. Strictly speaking, the fundamental equation holds only for monochromatic light, that is, light of a single wavelength. In the present case, however, the intensity ratio was measured over a small series of wave-lengths comprising about 4 μ. This is not accompanied by an appreciable error as long as the concentration and linear thickness are varied within narrow limits.

9. Martens and Grünbaum: Ann. d. Physik., 1903, iv, Series 4, 984.
10. Butterfield: Ztschr. f. physiol. Chem., 1909, lxii, 194.

The measurements were always made at two regions in the spectrum, (1) in green, at the maximum of the absorption band of oxyhemoglobin and (2), in yellow, at the minimum of absorption between the two absorption bands of oxyhemoglobin. The advantage of this procedure becomes apparent on combining the equations for the drop in the light intensities at two different wave-lengths for the same solution in the same linear thickness. Let the relations at one wave-length be represented by:

$$I_1' = I_1 e^{-k_1 dc}$$

and the corresponding relations at the other wave-length by:

$$I_2' = I_2 e^{-k_2 dc}$$

Then:

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}} = \frac{k_1}{k_2} = K$$

This affords a convenient and ready means of detecting variations in the spectrum of the blood under examination. It is possible to detect the presence of other derivatives of hemoglobin in the presence of oxyhemoglobin, which is of importance for the study of the probable nature of the color-index. The indices sub-one and sub-two denote in this paper a small spectral region at the maximum absorption of oxyhemoglobin in green and at the minimum in yellow respectively, k_1 and k_2 are the constants of oxyhemoglobin from ox-blood in these regions and have the values 19.4 and 11.7. Either one may be used for the determination of total hemoglobin concentration of the blood. In order to avoid confusion with other studies in which monochromatic light was employed and the same notation used,[11] it may be well to state again that in the following pages

$$\log \frac{I_1}{I_1} = \log \frac{I}{I'} \text{ at the maximum of absorption in green, and}$$

$$\log \frac{I_2}{I_2'} = \log \frac{I}{I'} \text{ at the minimum in yellow.}$$

For the spectrophotometric measurements the blood was laked in all cases with distilled water. The dilutions were prepared by emptying the contents of an Ostwald pyknometer into volumetric flasks.

Under the conditions of the measurements, the light absorption of the serum is negligible, as was first established by Vierordt. This point has been repeatedly checked with normal and pathological serums in a 2 cm. layer.

Hemoglobin Determination by the Sahli Method.—In most cases determinations were also made with the Sahli apparatus. In such cases the final reading was not taken until the maximum browning of the aqueous blood and hydrochloric acid mixture had been reached. This sometimes required twenty-four hours.

Specific Gravity.—The specific gravity was determined with an Ostwald pyknometer, weighing first when empty, then when filled with blood, and finally, with distilled water at a known temperature.

The accompanying table gives the results in full.

11. Butterfield: Proc. Soc. Exper. Med. and Biol., 1913, x, 74; Butterfield and Peabody: Jour. Exper. Med., 1913, xvii, 587.

It will be seen at once that the extreme variation for the color-index of normal blood in our cases is from 0.9 to 1.1. In pernicious anemia the index is markedly raised, as is also the average hemoglobin content

TABLE 1.—Hemoglobin Determinations in Blood from Normal and from Pathologic Subjects

	Specific Gravity	Sahli Reading	Erythrocytes Millions per cu.mm.	$\frac{\text{Log}\frac{I_1}{I'_1}}{\text{Log}\frac{I_2}{I'_2}}$	Hemoglobin Content of Blood gm. per 100 c.c.	Quantity of Hemoglobin in Average Erythrocyte, gm. $\times 10^{-11}$	Color Index
NORMAL BLOOD							
Men, 1	1.057	95	4.29	1.66	15.4	3.6	1.1
2	1.055	77	4.19	1.65	14.3	3.4	1.0
3	1.062	100	5.19	1.67	17.4	3.2	0.9
4	1.061	97	5.46	1.68	17.8	3.3	1.0
5	1.056	87	4.57	1.64	15.3	3.4	1.0
6	1.066	105	5.79	1.65	19.3	3.3	1.0
7	1.060	93	4.74	1.65	16.9	3.6	1.1
Averages	1.059	93	4.92	1.66	16.6	3.4	1.0
Women, 1	1.057	83	4.42	1.64	15.8	3.6	1.1
2	1.058	88	5.11	1.64	15.6	3.1	1.0
3	1.053	77	4.44	1.67	13.7	3.1	1.0
4	1.061	97	5.11	1.64	17.0	3.3	1.0
5	1.055	85	4.46	1.64	13.7	3.1	1.0
6	1.056	90	4.95	1.65	15.2	3.1	1.0
Averages	1.057	87	4.75	1.65	15.2	3.2	1.0
PATHOLOGICAL CASES							
Pernicious anemia I	1.040	21	0.74	1.65	3.47	4.7	1.5
" " II	1.035	23	0.87	1.65	3.79	4.4	1.3
Secondary anemia..	—	—	2.43	1.63	5.59	2.3	0.7
Polycythemia	1.075	—	—	1.66	23.9	—	—

of the red blood-corpuscles. In secondary anemia a low index is found. Unfortunately, chlorosis was not included in the present series. In all probability, the values here presented for pernicious anemia and secondary anemia do not represent the extreme possible variations.

From the general agreement with Laache's results, however, there seems to be no reason for hesitating to accept his extreme values of 0.4 to 2.3.

Passing to the absolute values, it will be noticed that the average hemoglobin content of normal blood is considerably higher than the accepted value of 14 gm. in 100 c.c. of blood. There are two reasons for this. In the first place, defibrinated blood was used in all cases in the present work, and it is possible that there is a slight decrease in volume after removal of the fibrin. In the second place, the old value of 14 gm. in 100 c.c. dates back chiefly to Otto's[12] determinations with the old model of the Hüfner spectrophotometer, using the optical constant of oxyhemoglobin from dog's blood. It is not permissible to employ the constant of dog hemoglobin for determinations on human blood, unless one has previously established the identity of the optical constants for both sorts of hemoglobin. As has been mentioned, this was done in the case of oxyhemoglobin from human blood and from ox-blood. If the determination of the constants has been carried out with the proper precautions, it would seem to be immaterial what spectrophotometer is used, provided, of course, that the construction and adjustment are correct. For example, we found in Tübingen with a new model of the Hüfner spectrophotometer, a hemoglobin content of 16.3 gm. in 100 c.c. of the mixed defibrinated blood from three healthy men, a value which is in close agreement with the average for men in Munich, determined with spectrophotometer of König, Martens and Grünbaum, that is, 16.6 gm. in 100 c.c.

Especial emphasis is to be laid on the constancy of

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$$

for normal human blood and for the blood in pernicious anemia, polycythemia and secondary anemia, which goes to prove that in these conditions there is no constitutive change in the oxyhemoglobin molecule which can be detected optically. From previous work it also follows that little or no other change is probable, inasmuch as the oxygen capacity and the iron content are also constant per gram hemoglobin in the conditions mentioned and in chlorosis.

12. Otto: Arch. f. Physiol. (Pflüger's), 1885, xxxvi, 36.

NATURE OF THE HIGH COLOR-INDEX

Since the color-index was first observed, up to the present time, various explanations have been advanced. It was first suggested that the high color-index was only apparent — that a number of small erythrocytes were either broken up and dissolved in the diluting fluid or missed in the counting. Laache was inclined to believe that in some cases the high color-index could be accounted for by the number of macrocytes. In fact, in going over his figures one finds in some of his cases that the color-index is almost proportional to the square of the average radius of the erythrocytes. The same is true in the work of Capps[13] on the volume-index, which he found to follow closely the color-index. This explanation by the increase in the total volume of the erythrocytes seems to be the most probable. It is really immaterial for the present, however, whether the phenomenon is due solely to an actual increase in the volume of the erythrocytes with constant intracellular hemoglobin concentration, or at the same time partially due to an increase in the hemoglobin concentration of some of the erythrocytes. The question of primary importance may be stated in the following terms. Given a blood with a high color-index and one with a low color-index, but with the same total hemoglobin concentration: Does the distribution of the same quantity of hemoglobin over a different number of particles, that is, red blood-corpuscles, affect the oxygen capacity of the native blood? This can be settled only with suspensions of red blood-corpuscles from the essential blood-diseases, and almost all of the previous work has been done with laked blood.[14]

Recently, Pappenheim[15] has advanced the view that the high color-index is due to a true *hyperchromatism* of the erythrocytes. He does not regard this in the light of an increase in the hemoglobin concentration within the erythrocyte, but assumes a degeneration (?) of the blood coloring-matter with the formation of methemoglobin or promethemoglobin (?). There is absolutely no evidence in support of such a view as far as pernicious anemia of human pathology is con-

13. Capps: Jour. Med. Research, 1903-1904, v, 367.

14. Experiments with suspensions of red blood-corpuscles are in progress. The method of Paal, which consists in reduction with colloidal palladium in an atmosphere of hydrogen, is well suited to this work. Experiments with normal red blood-corpuscles suspended in salt solution have already been carried out. It has been found that the oxygen content of suspensions of normal red blood-corpuscles is proportional to the total hemoglobin concentration. Two suspensions have been used. One contained red blood-corpuscles corresponding in number to normal blood, while the other was ten times more dilute.—*Butterfield.*

15. Pappenheim: Fol. Haematol., 1911-1912, xii, Part 2, p. 68.

cerned.[16] In fact, there is conclusive evidence against this view. We mentioned in the beginning that the oxyhemoglobin in pernicious anemia is identical with that of normal blood, as far as the light absorption, gas absorption and iron content are concerned. That the high color-index should be caused by methemoglobin is contrary to actual facts. With the exception of the band in red, methemoglobin in neutral solution absorbs in yellow, green and violet less light than oxyhemoglobin.[11] When the colorimetric power of the blood is determined, as hematin with the Sahli apparatus, it is possible that methemoglobin may give an apparently higher reading than oxyhemoglobin, but only when the reading is made shortly after adding the hydrochloric acid and diluting. If the determination is made properly, that is, the reading taken after awaiting the maximum browning of the solution, the presence of methemoglobin should not influence the Sahli method. As far as direct measurements of the light absorption are concerned, the presence of methemoglobin would give an apparent decrease in the oxyhemoglobin content, if the measurements were made in yellow, green or violet; exactly the opposite of Pappenheim's contention.

THE COLOR OF THE RED BLOOD-CORPUSCLES

The study of the light absorption of oxyhemoglobin leads naturally to a discussion of the color of the red blood-corpuscles. It is well

16. Pappenheim[35] bases this view on the assumption that the chemicals which are used for the production of experimenttal anemia are at the same time substances which convert oxyhemoglobin into methemoglobin. As far as we are aware, however, a high color-index in the sense of Laache has never been observed in experimental anemia, toxic or posthemorrhagic. His "proof" of his view is as follows: 1. One determines the coloring-power of a portion of centrifuged and washed sheep corpuscles by the Sahli method. 2. To an equal portion of the washed corpuscles is added hydroxylamin, pyrodin, or toluylenediamin dissolved in a drop of physiological salt solution or distilled water. The coloring-power is then determined by the Sahli method. It will be found that the Sahli reading in the second case is almost double that of the first. This is not at all remarkable when one considers that the Sahli method rests on the conversion of oxyhemoglobin into hematin and subsequent comparison with a "standard" hematin solution. The conversion of oxyhemoglobin to hematin requires time, especially with the Sahli technic (tenth-normal hydrochloric acid at room temperature). It will be found that if one takes a Sahli reading immediately after adding hydrochloric acid and diluting with water up to apparent equality with the "standard" solution, and then lets the preparation stand twenty-four hours, it will often be necessary to dilute further, so that the final reading is often 20 per cent. higher than the initial reading. On the other hand, if methemoglobin is present, the brown color of methemoglobin is much nearer that of hematin, so that in the gradual conversion to hematin, the maximum browning may be reached sooner than in starting from pure oxyhemoglobin. In all cases before complete conversion has been accomplished, one is comparing a mixture of oxyhemoglobin, methemoglobin, and hematin with the "standard" hematin solution. Hence the confusion. Taking the proper precautions, one of us (*B.*) has repeated the Pappenheim experiment with a completely negative result.

known that single corpuscles viewed in transmitted daylight are light green or yellowish green in color. The red tinge does not become noticeable until transmitted light traverses three or four superposed corpuscles. As far as we know, a satisfactory explanation of this phenomenon has never been advanced. A comparison of the spectra of the greenish single layer and of the reddish superposed layers gives, we believe, the clue to the explanation of the phenomenon. It will be observed that there are two minima of absorption, that is, two maxima of intensity of the transmitted light, one in red and the other in yellowish green. On increasing the thickness of the absorbing layer, that is, the number of superposed corpuscles, the intensity of the transmitted light at the absorption minimum in yellowish green diminishes abruptly, while the diminution of intensity in red is inappreciable. This means that the constant of light absorption at the minimum in green is much greater than the corresponding constant at the minimum in red, in symbols $k_{gr} > k_r$. If we regard the intensities in these two regions only, assuming that the absorption in the other regions of the spectrum is so great that the transmitted intensities are negligible,[17] then $I_{gr}e^{-k_{gr}lc} + I_re^{-k_rlc}$ would represent the intensity of the transmitted light.

Starting from a number of superposed corpuscles which appear red in transmitted light and decreasing progressively the number of corpuscles superposed, one would expect, in accordance with the principle of dichromatism,[18] that a color change from red to green would occur if the condition, $I_{gr}e^{-k_{gr}lc}$ equal to or greater than $I_re^{-k_rlc}$, could be realized. In the case of red blood-corpuscles viewed in transmitted daylight the necessary conditions, $I_{gr} > I_r$, $k_{gr} > k_r$, and l sufficiently small, are all favorable to the realization of $I_{gr}e^{-k_{gr}lc} > I_re^{-k_rlc}$. It would be reasoning in a circle to say that this is a demonstration of the cause of the green color of a single layer of red blood-corpuscles. Even if it were found that for the correct values of I_{gr}, I_r, k_{gr}, and k_r, the observed l, at which the color change occurred, agreed with the calculated, then the demonstration would not be complete, for one must account for the sum of all intensities in the transmitted light, and

17. Strictly speaking, one should formulate for the intensities over the whole visible spectrum. This leads to an integral which has no practical significance as long as we do not know what functions I and k are of λ (the wave-length). See remarks of S. P. Langley: Am. Jour. Sci., 1884, Third Series, xxviii, 168.

18. Dichromatism must not be confused with dichroism. Dichromatism depends on the selective absorption of isotropic substances, while dichroism is a special case of pleochroism, which refers to anisotropic crystals in which the light absorption varies with the direction of the vibrations of the light waves.

this leads to difficulties, mathematical and experimental, which are at present insurmountable.

There remains, however, the possibility of a demonstration by exclusion. In the first place, it is possible to exclude the effect of the stroma and of the surface curvature on the color of the red blood-corpuscles. A very thin wedge of solid oxyhemoglobin can be made by allowing a drop of a concentrated solution of oxyhemoglobin to evaporate under a cover-glass 6 cm. long, of which one end is very slightly raised. The linear thickness of different parts of such a wedge can be estimated from the interference phenomena which are visible in the spaces unoccupied by oxyhemoglobin, between the cover-glass and slide. At 0.2 microns the oxyhemoglobin is colorless, between 0.2 microns and 1.3 microns the oxyhemoglobin is yellowish green, that is, of the same color as red blood-corpuscles in a single layer, while above 1.3 microns the reddish tinge is noticeable and increases with the thickness of the wedge to a vivid red. The color change is the same with illumination by a bundle of parallel rays and with illumination by bundles with different degrees of convergence. This, again, is not a rigid proof of the dichromatism of red blood-corpuscles, but it does prove that the color change observed with red blood-corpuscles may be perfectly imitated with a homogeneous isotropic layer of oxyhemoglobin of varying thickness.[19] This, taken in conjunction with the spectroscopic appearances, eliminates dichroism, surface reflection and interference as necessary factors for the explanation of the color of the red blood-corpuscles, and leaves by exclusion the dichromatism of oxyhemoglobin as the most probable explanation of the phenomenon.

CONCLUSIONS

1. The existence of a high color-index in pernicious anemia has been established with exact methods. The high color-index is due to an increase in the oxyhemoglobin content of the erythrocytes. A quantitative study of the light absorption shows that oxyhemoglobin alone and no other derivative of hemoglobin is present in the blood of cases of pernicious anemia with a high color-index.

2. The color-index of normal human blood varies within the narrow limits of 0.9 to 1.1. The average total hemoglobin concentration of normal defibrinated human blood is in the neighborhood of 16 gm. in 100 c.c. The color of the red blood-corpuscles is best explained by the principle of dichromatism as applied to the absorption spectrum of oxyhemoglobin.

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19. It may be mentioned, incidentally, that very small (microscopic) crystals of oxyhemoglobin seem to be identical in color with the red blood-corpuscles, while larger crystals are bright red.

HUMAN STREPTOTRICHOSIS AND ITS DIFFERENTIATION FROM TUBERCULOSIS*

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Members of the somewhat ill-defined group of micro-organisms, *Streptothrix*, produce the disease streptotrichosis in man and animals. There is a gradually increasing recognition of the close relationship between these organisms and the bacillus of tuberculosis; this, together with an equal similarity between these two diseases, focuses our interest sharply on the problem of their prompt recognition and early differentiation from each other.

The literature of the subject is extensive, and to be found chiefly in periodicals. A few of the large handbooks of medicine, Osler's and Allbutt's, give clear discussion of the mold fungi, as to their structure and as agents in disease production; but on the whole the usual medical text-books pass the subject casually, so that the student of medicine has no very good idea of the biological characters of, and relations between the streptotriches, sporotriches and blastomycetes, or of their really important rôle in the production of disease in man and animals.

The observations of these fungi made by Mayen, 1827; Corda, 1842; Langenbeck, 1845, and others are our earliest records. Carter from 1859 to 1874 first clearly proved the pathogenic rôle of streptotriches in man, his work centering on Madura foot. The classic work of Bollinger[1] (1877), Hartz[2] (1878), and Israel[3] (1878) established the recognition of the disease and its cause in both man and cattle. Boström[4] (1891) completed an exhaustive laboratory study of the organisms isolated from eleven cases of streptotrichosis. Wolff and Israel did likewise in the same year. Many others have added to our knowledge of these fungi and their lesions, the greater part of the published material being, however, more or less complete case reports

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1. Bollinger, O.: Ueber eine neue Pilzkrankheit beim Rinde, Centralbl. f. med. Wissensch., 1877, xv, 481.
2. Harz, C. O.: Actinomyces bovis, ein neurer Schimmel in den Geweben des Rindes, Jahresb. d. k. Centr. Thierarzneischule in München, 1877-8, p. 125.
3. Israel, J.: Neue Beobachtungen auf dem Gebiete der Mykosen des Menschen, Virchows Arch. f. path. Anat., 1878, lxxiv, 15.
4. Boström: Demonstration mikroskopischer Präparate von Aktinomykose der Menschen und Thiere, Berl. klin. Wchnschr., 1885, xxii, 14, 274.

with various cultural data, some purely biological and some including experimental studies, with a few articles giving a well-balanced critique of the whole subject. The last-mentioned serve to show how much a provisional basis of classification and a working hypothesis are needed for this group, so that observations may be comparative. In recent years Vincent,[5] Wright[6] and especially Foulerton[7] have done much to clear away the confusion surrounding these organisms. Musgrave, Clegg and Polk[8] offer a bibliography of over 1,500 references as well as a good historical summary, a critical biological study of six species and some significant experimental data on monkeys. Their work had especial reference to the various forms of Madura foot.

In this mass of facts, clinical, biological and pathological, there are several that stand out prominently. The appearance of the disease is in distinct types; as a rule slow, progressive, markedly local, but capable of an acute, rapid general pyemic or pneumonic course, with a fatal termination in from one to three weeks. These cases may be especially confusing, since little or no discharge is present in which to find the organisms, and probably these are in a bacillary or rod-segment stage, not the fungoid threads and granules usually encountered and looked for. A slow chronic case may thus flare up and terminate fatally; especially is this noted in secondary cerebral involvement following lung or bone infection, making a complete analogy to tuberculous meningitis.

The confusion between tuberculosis and streptotrichosis is well illustrated by many writers in their reports on pseudotuberculosis and pseudo-actinomycosis. The rôle played by acid-fast and bacillary streptotriches is here prominent, still further masking the nature of the infection and so allying it apparently to tuberculosis. The use of carbolfuchsin as a stain is a further cause for confusion, since usually only the acid-fast parts of the streptothrix are thus demonstrated, while with the Gram method both the acid-fast and non-acid-fast elements stain equally well. One of the earlier descriptions of these acid-fast types is Nocard's[9] original discussion of *Streptothrix*

5. Vincent, H.: Etude sur le parasite du "pied de Madura," Ann. de l'Inst. Pasteur, 1894, viii, 129.

6. Wright, J. H.: The Biology of the Micro-Organism of Actinomycosis, Jour. Med. Research, 1904, xiii, 349.

7. Foulerton, A. F. G.: Streptothrix Infections, Lancet, London, 1899, ii, 779; Pathology of Streptothrix Infections, ibid., 1906, i, 970; Streptotrichosis and Its Relation to Tuberculosis, ibid., 1910, i, 551, 626, 771; Some Observations on a Series of Seventy-Eight Cases of Streptothrix Infections, ibid., 1913, i, 381.

8. Musgrave, Clegg and Polk: Streptotrichosis, with Especial Reference to the Etiology and Classification of Mycetoma, 1903, iii, 447.

9. Nocard: Note sur la maladie des bœufs de la Guadeloupe, Ann. de l'Inst. Pasteur, 1888, ii, 293.

nocardii (farcinica), an acid-fast strain that causes *farcin du boeuf*, a disease suspected of being tuberculosis until Nocard's clear demonstration that the organism was an acid-fast streptothrix. In laboratory cultures the acid-fastness is but partial; only certain of the individual rods remain red, the rest taking the counter-stain. In 1898 Flexner[10] discussed under the name "pseudotuberculosis" a case of a man aged 70 with both lungs extensively consolidated and the clinical symptoms of tuberculosis. No sputum was obtained, the diagnosis being made post mortem. No tubercle bacilli, but faintly red-staining, branched organisms with no bacillary forms, were found in smears of lung, and a slightly different form, more bacillary, from peritoneal nodules. Cultures showed no growth. An article in the same year by Berestneff[11] on pseudo-actinomycosis drew attention to the similarity of its pathology to that of actinomycosis and the absence of the granules and clubs then supposed to be essential to actinomycotic infection. Lubarsch[12] saw the relationship of *Bacillus tuberculosis* and its nearly related acid-fast neighbors to the acid-fast and non-acid-fast streptotriches, and the similarity of the diseases which they produce.

Feistmantel,[13] after careful experiment and critical observation of *Streptothrix nocardii (farcinica)* as to its acid-fastness and variations in structure in tissues, asserted its close relation to the acid-fast organisms on the one side and actinomyces on the other, calling them all *Streptothrix*. Bert and Leishmann[14] reported a pulmonary infection of a dragoon at Ladysmith, South Africa, with an acid-fast streptothrix showing branched and unbranched rods. The organism in pure culture was obtained from the lung and pericardial fluid at necropsy. In the same year Ophüls[15] reported on five cases of pulmonary gangrene in California caused by acid-fast, branched bacilli from which pure cultures were not obtained. He considers that the bacteria belong probably to the streptothrix group associated with pyogenic cocci in causing the disease. Sanfelice[16] and Galli-Vallerio[17] have also given

10. Flexner, S.: Pseudo-Tuberculosis Hominis Streptothrica, Jour. Exper. Med., 1898, iii, 435.
11. Berestneff, N.: Ueber Pseudo-Aktinomykose, Ztschr. f. Hyg., 1898, xxix, 94.
12. Lubarsch: Zur Kenntniss der Strahlenpilz, Ztschr. f. Hyg., 1899, xxxi, 185.
13. Feistmantel: Saure- und Alkohol-Festigkeit der Streptothrix farcinica und die Beziehungen der Streptotricheen zu den Saurefesten Pilzen, Centralbl. f. Bakteriol., Orig., 1902, xxxi, 433.
14. Bert and Leishmann: A New Acid-Fast Streptothrix Pathogenic to Man and Animals, Jour. Hyg., 1902, ii, 120.
15. Ophüls: Acid-Proof Bacilli in Five Cases of Pulmonary Gangrene, Jour. Med. Research, 1902, viii, 242.
16. Sanfelice: Streptothrix-Pseudotuberculose, Centralbl. f. Bakteriol., Orig., 1905, xxxviii, 30.
17. Galli-Vallerio: Etudes sur les Actinomycetes, Centralbl. f. Bakteriol., Orig., 1912, lxiii, 555.

further evidence as to the pseudotuberculoses caused by streptotriches, and the close relationship between them and the whole group of acid-fast organisms. This property of acid-fastness is, however, a variable one; it may be considered a function rather than a structure, since it can be assumed or discarded by the organisms apparently according to circumstances; for example, the butter bacillus gradually assumes acid-fastness when lying in the fat, losing it again when removed to a fatless medium. Sanfelice suggests that the oil in the skin of those parts of the body normally associated with acid-fast organisms assists in developing this property. Some remarkable work has been done in the last few years on the organism of leprosy. Williams[18] has isolated from leprous nodules three types, a non-acid-fast mycelium and non-acid-fast diphtheroid organism, and an acid-fast bacillary and mycelial form; both the former under appropriate conditions develop acid-fast properties, the diphtheroid forms doing so in forty-eight hours when devoured by amebas; those simply lying near the protozoa may develop some acid-fast granules. The acid-fast rods disappeared after the third subculture, and with appropriate subcultures the organisms again assumed acid-fastness. These observations have been corroborated by other writers. Williams used a vaccine made from the acid-fast cultures, which called out an apparent specific reaction on injection into lepers. The non-acid-fast type failed to do this, but from past experience in serum reactions it seemed necessary to use specially efficient endotoxin extraction (Besredka's, Gay's modification) for antigens in the mycelial types. Reactions would probably occur if such preparations were used. The remarkable pleomorphism of this group of organisms is well illustrated in these results, and the great possibility of confusion and misapprehension as to the character of a strain isolated from any given infection.

The susceptibility and frequent infection of cattle is worthy of consideration; in fact, better discussions of this disease and its causal agents can be found in veterinary than in human text-books on pathology. Acute infections occur as well as the classic lumpy jaw. Kieffer[19] reports an outbreak of bovine pneumonia in a small dairy, five cows dying in a few days. A freely growing streptothrix was isolated from the blood and all viscera examined. Usually these bovine infections are caused by the well-recognized *Streptothrix bovis*, but the relation, if any, between the organisms in the slow chronic cases and rapidly fatal pneumonias is not established. With pleomorphic organisms, such as these streptotriches, it is to be borne in

18. Williams, T. S. B.: Leprosy: A New View of Its Bacteriology and Treatment, Brit. Med. Jour., 1911, ii, 1582.

19. Kieffer, C. F.: A New Streptothrix Pathogenic to Cattle, Med. Rec., New York, 1907, lxxi, 346.

mind that a variation in pathogenicity due to changes in host, parasite or both can readily allow one morphological form to predominate. There is reason to think that the extension of the disease both in the diseased organ and from case to case is made by the rod segments or bacillary forms; they migrate, take root, as it were, forming a new plant which grows and casts off in turn these readily disseminated structures. It is a moot question whether streptotrichosis in man is caused by the same species of streptothrix which occurs in cattle. Foulerton, from his observations, believes the human cases to be only occasionally produced by *Streptothrix bovis,* some form of *Streptothrix hominis* or the partly acid-fast types being the usual agents. The necessity for a clear determination of specific characters and their limits of variability is emphatically demonstrated here.

There is an almost entire failure of early diagnosis in streptotrichosis. The impression gained from the case records is of an overwhelming mortality. Relatively few cures are recorded, largely because diagnosis is not made till a late stage, or even after death. In infections of the skin, it is more easily made early, and such cases afford the majority of cures. Early slight infections, perhaps spontaneously curable, pass unrecognized, and hence unrecorded.

There seems to be no marked geographical distribution of this disease, barring the tropical Madura foot, which, being essentially a wound infection, occurs particularly among the barefoot races. In Russia[20] there are reports of a large number of lung infections, whether owing to better diagnosis or greater incidence of the disease, or to both causes, cannot be determined. Opokin states that it is frequently undiagnosed or called tuberculosis. A series of such infections, chiefly of the lungs, was reported from California by Bridge.[21] They were found among a large number of presumably tuberculous cases. Captain Kremer, one of the Army medical staff stationed at Honolulu, speaks of the occurrences of these fungoid infections among the residents of the islands.[22] Whatever are the conditions, and they are probably complex, that lead to the frequency of the disease in the mild semitropical climate of the Hawaiian Islands, the moderate one of California, north and south, and the comparatively frigid Russia, this wide range shows the problem of these infections to be no small one. The frequent confusing clinical similarity to tuberculosis only adds emphasis to these facts. All clinicians are familiar with so-called tuberculous lung infections, without demonstrable bacilli, and it is

20. Opokin, A. A.: Lungen- und Pluerenaktinomykose nach den Beobachtungen russischer Autoren, Arch. f. klin. Chir., 1909, lxxxviii, 319.
21. Bridge, Norman: Streptotrichosis (Actinomycosis) of the Lungs, Jour. Am. Med. Assn., 1911, lvii, 1501.
22. Kremer: Personal letter to author.

among such, especially, that cases of streptotrichosis are to be found.

The streptotriches, non-acid-fast, partly acid-fast, and wholly acid-fast, have long been associated with herbage quite as intimately as the well known acid-fast organisms allied to *B. tuberculosis*. Both saprophytic and pathogenic strains have been isolated from soil, water and foodstuffs. They are omnipresent. Petruschky[23] investigated the carpets in many rooms of an old public building in Danzig and demonstrated several strains near the windows; one strain was pathogenic to guinea-pigs. (This investigation followed the recognition of an infection in one of the men employed in the building.) Not only can they be found in an apparently saprophytic condition outside the body, but it is well known that certain apparently harmless strains can be demonstrated in the mouth and nasopharynx of man. Lord[24] has made some suggestive observations on the cheesy contents of the crypts of enlarged tonsils and also the material obtained from dental caries. In smears and in serial sections from both he obtained a typical Gram-positive streptothrix. Peritoneal inoculation of guinea-pigs yielded characteristic lesions with colonies of a branching organism in 60 per cent. of the animals examined at necropsy from twenty-one to forty-five days after injection. Lord believes that there is an etiological relation between it and the diseased teeth and enlarged tonsils. So-called non-pathogenic forms are frequently present in the mouth, particularly when any abnormal condition exists, as pyorrhea alveolaris, or otherwise abnormal teeth and gums.

With these facts in mind, data as to the location of streptotrichosis in the body assume greater significance. The percentage of cases occurring in the head and neck, as gathered from three divergent sources, is summarized in Table 1.

TABLE 1.—HEAD AND NECK INFECTIONS WITH STREPTOTRICHES

	No. Cases	Per Cent. Cases
Foulerton (Middlesex hospital) London	78	51.0
Acland (British)	109	38.5
Ruhräh (American, collected cases)	632	57.0
Average		49.0

An average of 49 per cent., nearly half the recorded cases, involve the head and neck region, near which organisms with supposedly small, if any, pathogenic powers are frequently to be found. The slightly abnormal state of inflamed gums, enlarged tonsils, and decayed teeth, however, can readily offer the *locus minoris resistentiae* so essential to infection. Here also is emphasized the confusion possible with

23. Petruschky: Kolle und Wassermann's Mikroorganismen, 1912, v, 280, etc.

24. Lord, F. T.: Actinomycosis: Its Production in Guinea-Pigs from Carious Teeth, Boston Med. and Surg. Jour., 1910, clxiii, 82; The Etiology of Actinomycosis, Jour. Am. Med. Assn., 1910, lv, 1261.

tuberculosis. There is nothing characteristic in the clinical appearance of abscesses or enlarged glands, or in fact of any form of streptotrichosis that differentiates it clearly. The recognition of the parasite in discharges is frequently difficult, and in many cases, especially early in the trouble, there is no discharge to examine. This is markedly so in cervical adenitis, usually assumed to be tuberculosis, in which only a careful examination of the removed glands in culture and section can determine the true nature of the disease in early stages. Burnet[25] reports an examination of twenty-three cases of cervical adenitis in children and adults that were clinically tuberculous; twenty-two yielded cultures of *B. tuberculosis* on Dorset's egg-medium; one a pure culture of a filamentous, branched organism, Gram-positive, non-acid-fast, producing red colonies, quite probably *Streptothrix rubra,* a species known to be at times pathogenic. It was obtained from the pulp of the gland. The latter was the size of a small egg, non-caseous, non-calcified with a consistence like a very firm carcinoma. A similar case came under my personal observation and will be reported on later.

Records of chest infections are numerous, averaging about 18 per cent. of all cases of streptotrichosis. With greater accuracy of observation, this percentage would doubtless be increased. There are also a significant number of appendix infections, as Foulerton has especially pointed out. Of his seventy-eight cases twenty were of the appendix, with six deaths, due in four cases to suppurative thrombosis of the portal vein.

The need of some more ready and accurate means of diagnosis is evident. Clinical symptoms are not distinctive. The pathologist may make a diagnosis from discharges or tissues, examining them for gross granules or microscopic fragments by using the Gram stain, provided the lesions are not accepted as tuberculosis. Cultures are possible in some cases, but the well-recognized difficulty in the cultivation of some strains makes this method only partially successful. There remains the large and most important group of early cases without discharge and of those so deep seated as to be unavailable. The necessity of differentiation from tuberculosis is always present and rarely considered.

The results of a recent experimental study[26] of a series of these organisms and their serological reactions in immunized rabbits adds further evidence for the near relationship between the streptotriches and *B. tuberculosis.* The various partly acid-fast species are links connecting the mycelial, entirely non-acid-fast strains at one end with

25. Burnet, E.: Streptothricee dans une adénopathie cervicale, Compt. rend. Soc. de biol., 1913, lxxiv, 674.

26. Claypole, E. J.: Classification of the Streptotriches, Particularly in Their Relation to Bacteria, Jour. Exper. Med., 1913, xvii, 99.

the entirely acid-fast *B. tuberculosis* and its allies at the other. The morphological variability and many of the cultural peculiarities common to these organisms and to the small but important group of the acid-fast bacilli also emphasize this community of stock. The mere fact of acid-fastness is no absolute criterion since there is experimental evidence (Williams,[18] Marino,[27]) to show that many species assume or discard it according to circumstances, the condition being somewhat analogous to the spore state in other bacteria, which offers more resistance to unfavorable surroundings. In the serological reactions referred to, the two end members of this series were clear cut from each other; while the intermediate members reacted with both extremes, the latter would not interact. It was this relation that suggested the preparation of glycerinated extracts, similar to Koch's old tuberculin, and their use in skin tests.

STREPTOTRICHINS AND THEIR PREPARATION

Streptothrix hominis III (Foulerton) and *Streptothrix eppingeri* were selected, respectively, to represent the non-acid-fast mycelial and the partly acid-fast bacillary strains. The method of preparation of the streptotrichins is as follows:

Flasks of 5 per cent. glycerin bouillon were planted with the cultures and incubated at 37 C. In a week or two *Streptothrix eppingeri* began to form a floating, crinkled, bright orange membrane that heaped up in a manner similar to the characteristic growth of *B. tuberculosis*. This was allowed to grow for six weeks in Culture 1, and made streptothricin E. It is more difficult to make the *Streptothrix hominis* type float, but the addition of cork islands, on which the fragment of mycelium was originally placed, assisted materially in supporting a film. Culture 2 of this strain was grown for only two weeks, owing to the lack of successful film formation and the consequent drowning of the organism. From this, streptotrichin H was made. Preparations of the streptotrichins were made as follows, from Cultures 1 and 2:

They were heated one hour at 60 C., filtered through a porcelain filter and concentrated to one-tenth of their original volume, making 60 per cent. glycerinated extracts. These two streptotrichins, Koch's old tuberculin (O. T.) and a control of plain glycerin bouillon concentrated to one-tenth volume were used in the cases reported.

The skin test was performed in 100 cases, 55 abnormal persons and 45 controls, the latter presumably with no active disease of the sort under investigation; a few were convalescent from acute illness, some under surgical treatment, and 16 were children of from 3½

27. Marino, F.: Atténuation de la virulence du bacilli tuberculeux dans le tube digestif des hirudinées, Compt. Rend. Soc. de Biol., 1911, lxxi, 220.

months to 11 years of age. Among the 45 controls, there were 22 tuberculin reactions and no streptotrichin reactions. These tuberculin reactions appeared in 17 adults and in 5 children, without clinical symptoms, but some with records of cases of tuberculosis in the family or old suspicious illness from which there was complete recovery. In the group of 55 abnormal cases, 36 positive and 1 doubtful tuberculin reactions were observed, 13 streptotrichin-H reactions, 8 streptotrichin-E reactions, and 6 double reactions. Forty-two of these cases had lung infections frankly tuberculous or only clinically so; 11 were gland cases, and 5 bone infections. Details of these cases and their reactions are given in Table 2. The skin tests were all made with a von Pirquet chisel, avoiding trauma as much as possible; then sterile wooden toothpicks were dipped into the respective preparations and the extract rubbed into the abrasions gently. They were allowed to dry for ten minutes, and examined at twenty-four- and forty-eight-hour intervals. There is a distinct difference between the reactions produced by the two streptotrichins. That of the party acid-fast streptotrichin-E is dense, indurated and long-lasting with an areola of from 6 to 10 mm., very similar to the typical tuberculin reaction, while the H-extract produces a more transient change very similar to that caused by the typhoidin used in the skin tests recently reported on by Gay and Force.[28] There is a characteristic areola from 6 to 10 mm. in diameter with a central induration that is quite perceptible to the touch. The reddened areola may be quite transient, disappearing in from twenty-four to forty-eight hours, while the induration may last several days, never, however, showing the dense nodosity so characteristic of the acid-fast reactions. It is apparent that the "tuberculonastin" and the fatty acids present in the tubercle bacillus or similar substances in the streptothrix produce a more intense local irritation. Such preparations have been shown by Deilmann[29] to cause more intense serum reactions than the non-acid-fast protein residue remaining after the extraction of these substances from the tubercle bacillus.

STREPTOTRICHIN-H REACTIONS

Of the 13 streptotrichin-H reactions, 11 were in cases of pulmonary disease and 2 were glandular infections. Of the lung cases 6 were advanced, 2 early of moderate extent, and 2 patients were recovering and had no sputum; 1 case was quite incipient. Seven gave the tuberculin test; 4 were negative. In the sputum of 9 of the 11, tubercle bacilli had never been demonstrated; in the remaining 2, acid-

28. Gay, F. P., and Force, J. N.: Preliminary Note on a Skin Reaction Indicating Protection Against Typhoid Fever, University of California Publications in Pathology, 1913, ii, 127.

29. Deilman, O.: Ueber die specifischen Stoffe des Tuberklebacillus und anderen Sauerefester Bacillen, Ztschr. f. Immunitätsforsch., Orig., 1911, x, 421.

fast organisms were demonstrated; Case 33 gave a clear tuberculin reaction in addition to streptotrichin-H; the other—Case 28—an equally distinct streptotrichin-E and no tuberculin reaction, indicating in all probability a double infection with a partly acid-fast and non-acid-fast strain, not with *B. tuberculosis,* as the sputum record would indicate. The granules of *Streptothrix hominis* were found in the sputum of all these cases except two from which no sputum could be obtained. In these two, Cases 30 and 32, all previous examinations of sputum had been negative for *B. tuberculosis.* In Cases 33 and 28, both in advanced stages, it is probable that the secondary invader was *Streptothrix hominis.* This seems to be undoubted evidence of the possibility of the coexistence of the two infections. There is no doubt great variation in the degree of acid-fastness possessed by these organisms, and the exact color limit of a given strain or even of individuals of a given strain will vary correspondingly, so that differentiation by tinctorial methods is impossible. In these skin reactions we are employing the whole product of the culture, the non-acid-fast protein as well as the acid-fast extracts. This should consequently be a more reliable basis for determining specific differences when the reaction is once established in the body than the tinctorial reactions of the micro-organisms which depend wholly on the acid-fast substances. The remaining two cases, 61 and 66, yielding a reaction to streptotrichin-H, were gland infections. The one, Patient 66, a child aged 6, had been operated on for adenitis, presumably tuberculous, two or three days before the test was applied. The patient gave a distinct reaction to streptotrichin-H, but none either to tuberculin or to streptotrichin-E. Six weeks before, large tonsils and adenoids had been removed successfully; no enlarged glands existed at the time of examination. One month later he returned to the hospital with a mass of enlarged glands on the left side. They were subsequently removed, the skin test being made a day or two later. The mass removed was about the size of a hen's egg, hard, discrete, and showed no fluctuation. On section of the tissues no distinct lesions of tuberculosis were evident; there were a few giant-cells but no bacilli or caseous softening, a good deal of round-cell infiltration, and marked density of structure. With the Gram stain rod-segments and fragments of threads of varying lengths were easily found, though none of the granules characteristic of older lesions were present. The case was acute and in the early stage of *invasion,* the tissues being quite generally attacked by the rod segments and threads of the fungus, and it is most probable that not sufficient time had elapsed to make the granules. The close connection between the removal of the enlarged tonsils and the infection of the glands is noteworthy; that the streptothrix was in the tonsillar crypts is most possible, and finding a good portal of entrance in the

TABLE 2.—Reactions in Fifty-Four Abnormal Cases *

No. of Patient	Lung Infection	Gland Infection	Bone Infection	Sputum Finding †	Skin Reactions			Remarks ‡
					O. T.	Strepto-trichin E.	Strepto-trichin H.	
1	.....	Cervical	.....	.....	+	0	0	
2	Moderate	.....	.....	T. B. +	+	0	0	
3	Moderate	.....	.....	T. B. +	+	0	0	
4	Advanced	.....	.....	T. B. +	0	0	0	
5	Advanced	.....	.....	T. B. +	0	0	0	Extensive nephritis.
6	Moderate	.....	.....	T. B. +	+	0	0	Both lungs.
7	Early	.....	.....	T. B. +	+	0	0	Rapidly advancing.
8	Advanced	.....	.....	T. B. +	0	0	0	Lung and larynx.
9	*Moderate*	*Scar in neck healed*		*T. B. +*	+	+	*o*	*Glands several years before.*
10	Advanced	.....	.....	T. B. +	+	0	0	
11	Moderate	.....	.....	T. B. +	+	0	0	
12	Moderate	.....	.....	T. B. +	+	0	0	
13	Advanced	.....	.....	T. B. +	0	0	0	
14	Incipient	.....	.....	T. B. +	+	0	0	
15	Very slight	Cervical	.....	No sputum	+	0	0	
16	Quiescent	.....	.....	No sputum	+	0	0	No record of T. B.
17	Moderate	.....	.....	Negative	+	0	0	
18	Slight	.....	.....	T. B. +	+	0	0	
19	Quiescent	.....	.....	T. B. 0	+	0	0	No record of T. B.
20	Moderate	.....	.....	T. B. 0	+	0	0	No record of T. B.
21	Moderate	.....	.....	T. B. +	+	0	0	
22	Advanced	.....	.....	T. B. +	+	0	0	
23	Moderate	.....	.....	T. B. +	+	0	0	
24	*Advanced*	.....	.....	*T. B. +*	*o*	+	*o*	*Markedly indurated.*
25	*Advanced*	.....	.....	*Negative*	*o*	+	*o*	*Large reaction.*
26	.....	.....	*Healed knee*	.....	*o*	+	*o*	*Childhood infection eight years before.*
27	*Moderate*	.....	.....	*Strept. H. +*	*o*	+	*o*	*T. B. never recorded.*
28	*Advanced*	.....	.....	*Strept. H. +*	*o*	+	+	*T. B. recorded in sputum.*
29	*Advanced*	.....	.....	*Strept. H. +*	+	*o*	+	*T. B. never recorded.*
30	*Recovering*	.....	.....	*No sputum*	*o*	*o*	+	*T. B. never recorded.*
31	*Slowly progressing*	.....	.....	*Negative*	*o*	+	*o*	*T. B. never recorded.*
32	*Recovering*	.....	.....	*No sputum*	+	*o*	+	*T. B. never recorded.*
33	*Advanced*	.....	.....	*T. B. and Strept. H. +*	+	*o*	+	*Abundance of both organisms.*
34	Moderate	.....	.....	T. B. +	+	0	0	
35	Slight	.....	.....	No sputum	+	0	0	
36	*Slight*	.....	.....	*Strept. H. + T. B. o*	*o*	*o*	+	*O. T. hypodermatically; twice o; asthma.*

TABLE 2.—Continued

No. of Patient	Lung Infection	Gland Infection	Bone Infection	Sputum Finding †	Skin Reactions			Remarks ‡
					O. T.	Streptotrichin E.	Streptotrichin H.	
37	*Moderate*	.....	.....	*Strept. H.* + *T. B. o*	*o*	*o*	+	*T. B. never found.*
38	*Early*	.....	.....	*Strept. H.* + *T. B. o*	*o*	*o*	+	*Six months; some signs.*
39	*Advanced*	.....	.....	*Strept. H.* + *T. B. o*	*o*	*o*	+	
51	.....	Mesenteric and peritoneal	.....	.....	?	0	0	Child; advanced.
53	.....	Cervical	.....	.....	+	0	0	Operation one week before.
54	Slight	Cervical	.....	T. B. +	+	0	0	Slight cough.
55	.....	.....	Kyphosis T. B.	.....	+	0	0	Since 2 years old; 10 years now.
61	.....	*Bronchial*	.....	*No sputum*	*o*	*o*	+	*Marked induration in skin; baby.*
62	.....	Mesenteric	.....	.....	+	0	0	
63	.....	Cervical	Cervical kyphosis	.....	+	0	0	
66	.....	*Cervical*	.....	.....	*o*	*o*	+	*Organisms in glands removed.*
69	.....	.....	*Dorsal kyphosis slight*	.....	*o*	+	*o*	*Obscure myelitis; slight kyphosis.*
71	Moderate	.....	.....	T. B. +	+	0	0	
72	*Advanced*	.....	.....	*T. B. o*	+	+	*o*	
74	Healed	.....	Dorsal kyphosis	.....	+	0	0	All active process over.
79	.....	.....	.....	.....	+	0	0	Fistula in ano.
89	*Incipient*	.....	.....	*Strept. H.* + *T. B. o*	+	*o*	+	*Slight signs.*
91	.....	Healed cervical infection	.....	.....	+	0	0	Eleven years before.
96	.....	Healed cervical infection	.....	.....	+	0	0	Glands removed three years ago.

* In this table + indicates a reaction, 0 indicates no reaction, T. B. = *B. tuberculosis*, and O. T. = Koch's old tuberculin. † From personal examination. ‡ From sputum reports from other records.

wound left by the first operation it rapidly invaded the lymph-glands. It was very regrettable that cultures were not made from the gland tissue before hardening.

Case 61 was that of a child of 13 months, admitted to the hospital with acute intestinal decomposition and a condition of malnutrition of seven months' standing. There was no recognizable lesion, but a roentgenogram showed very large bronchial glands. A distinct positive reaction to streptotrichin-H was given by this child; tuberculin and streptotrichin-E were both negative. Although unfortunately not possible of corroboration, this is presumptive evidence of a streptothrix infection. Case 89 was incipient; a slight cough and expectoration had lasted for six months, a slight loss in weight and a general below-par condition indicated some trouble. Tubercle bacilli had been looked for without result. The skin test gave a slight reaction to tuberculin and also a slight one to streptotrichin-H. The latter was not large, but quite recognizable. The patient was a dentist by profession and could in consequence have been subjected to repeated opportunities of infection.

STREPTOTRICHIN-E REACTIONS

Eight cases, 9, 24, 25, 26, 28, 31, 64 and 72, yielded a reaction to streptotrichin-E. Six were lung involvements, two bone diseases. Of the lung cases three were clear-cut: no tubercle bacilli in the sputum, no tuberculin reaction, and a definite streptotrichin-E reaction, without doubt infections with a less acid-fast organism than *B. tuberculosis*. Of the remaining three cases, two gave double reactions to tuberculin and streptotrichin-E. Of these one had tubercle bacilli in the sputum and scars of an old cervical gland trouble, while the other had no tubercle bacilli and was probably to be put in the same class as the first three. The sixth case gave a double reaction to the two streptotrichins and a negative reaction to tuberculin, with a record of tubercle bacilli and *Streptothrix hominis* in the sputum. Again, a more acid-fast streptothrix rather than true *B. tuberculosis* was probably the infecting agent.

Few data are available to show how long these reactions will last after recovery from infection. Case 91, streptotrichosis of neck eleven years before, with a negative reaction, is the only record. It may be relatively transient. The double reactions between either end and the middle member of our series might be looked on as group reactions (Feistmantel[30]) if it occurred invariably or if no corroborative evi-

30. Feistmantel: Die Tuberkulinreaktion Ein Beitrag zur Feststellung ihre Wesens als Gattungsreaktion, Centralbl. f. Bakteriol., 1904, xxxvi, 282, 406. Experimenting with guinea-pigs infected with *B. tuberculosis* and *Streptothrix nocardii*, Feistmantel found an equally severe reaction on injecting subcutaneously either O. T. or an analogous preparation from the *Streptothrix*. He concludes that the reaction is non-specific. It is to be remembered that his tests were subcutaneous and the doses of relatively large size.

dence were possible as to the nature of the infecting parasite. The tuberculin reaction in the absence of tubercle bacilli is of no value as evidence in favor of such infection, in view of its general occurrence in many apparently healthy cases. Of the two cases reacting to both tuberculin and streptotrichin-E, Case 72 had no record of *B. tuberculosis* in the sputum, while Case 9 had bacilli in the sputum with a recent lung and old gland involvement. The remaining case, No. 28, with both streptotrichin reactions, exhibited both acid-fast organisms and streptothrix granules in the sputum, with no tuberculin reaction. This was then probably a case of double infection with both organisms, a partly acid-fast and a non-acid-fast type. The latter seems especially likely to have been the secondary invader. It is not possible at present to clear away all doubts as to diagnosis in these cases of infection with the partly acid-fast forms, but it is of some assistance to be able to differentiate the end members, and evidence from these observations seems to justify this. Case 86 was a case of blastomycosis, and it is interesting to note that it was negative to streptotrichin and sporotrichin but positive to blastomycotin.

THERAPEUTICS

Three lines of treatment, singly or combined, have been applied to streptotrichosis: surgical removal of the infected part, vigorous internal use of some form of iodin or copper sulphate, and specific vaccine therapy. The surgeon unaided cannot hope to cure unless he makes an early diagnosis, since the spread of the organisms along lymphatics and blood-vessels may have occurred; only in cases sharply localized like gland infections or appendiceal cases can the whole area involved be removed. The markedly favorable influence of iodin on some cases of all fungoid infections, blastomycosis, streptotrichosis and sporotrichosis is well known, and especially is this true in the earlier, more curable stages. Some of the more advanced ones also respond significantly to it. Iodin in the form of potassium or sodium iodid, changing even to hydriodic acid when intolerance is exhibited, are the usual preparations. The amount taken should be increased daily until marked symptoms of iodism appear, and then either changed in form or reduced in amount and again increased; the presence of a maximum amount of iodin in the body is apparently unfavorable to the fungus. At first there is usually a noticeably larger amount of pus and sputum from the diseased area and a marked increase in the number of organisms present. This is followed by a reduction of both discharge and organisms, and, if the case is a curable one, of a gradual healing of the tissues. Copper sulphate in ⅓-grain doses three times daily has also been employed in the treatment of these cases. Like the iodids, it is liable to disturb the stomach, and it is doubtful whether

it has a specific effect on the fungus; but it is a useful alternate to the iodids.

Specific treatment in the form of vaccines yields variable results; but the number of cases observed and treated is too few to make a basis for safe generalization. Petruschky[31] in 1898 found a case of streptotrichosis in a schoolgirl aged 12, suspected of being tuberculous, whose mother and older sister had died of consumption, but who had no "demonstrable bacilli." A streptotrichin similar to Koch's tuberculin was prepared and used, and recovery followed. Cohn made a normal salt suspension of an agar-slant (1 c.c. to a culture), diluting 100 times. Of this he gave 0.3 c.c. subcutaneously with no local reaction, repeating an increasing dose on each third day, increasing 0.3 c.c. each time. The case was an advanced prostatic infection, one with many secondary disturbances and too poor a general condition to allow of any surgery. Under the treatment, the prostate was reduced to a small size, but the patient died from general causes. In 1911 Harbitz and Grondahl[32] attempted treatment with vaccines, with one recovery and one death. Their attempts to immunize animals were unsuccessful, all serum reactions being negative. It is probable that their preparations made from the cultures were defective. Normal salt suspension may not contain enough bacterial protein to call out immune bodies in experimental animals. An endotoxin extraction according to Besredka's and Gay's method[25] produces a highly successful preparation for this purpose. Williams[18] made a vaccine from the acid-fast type of *Streptothrix leproides,* which was at the time of writing (1911) proving quite successful in cases which had soft, "juicy" lesions with a good blood-supply. These lesions regressed noticeably, and fresh exacerbations and extensions of the disease were apparently checked. Ultimate cure Williams does not claim; time alone can demonstrate that fact. He thinks that the vaccine has better results than the nastin treatment of Deycke; having used both he is in a position to judge.

Kinnicutt and Mixter[33] used a vaccine in eight cases. It was made from autogenous cultures grown in bouillon, and when of sufficient size the masses were fished out and transferred to a sterile test-tube, broken up as much as possible with a glass rod, then heated to 60 C. for one hour, transferred to 50 c.c. of sterile salt solution and 0.1 c.c. used for first dose, repeated in three or four days with an increase of 0.05 or 0.1 c.c. each time. Eight cases were treated by Kinnicutt and

31. Petruschky: Demonstration von Preparaten und Kulturen von einem zweiten intra-vitam diagnostiziesten Falle von Streptotrichosis hominis, Deutsch. med. Wchnschr., 1898, xxiv, 78 v (Vereins-Beilage).

32. Harbitz, F., and Grondahl, N. B.: Actinomycosis in Norway: Studies in Etiology, Modes of Infection, Treatment, Am. Jour. Med. Sc., 1911, cxlii, 386.

33. Kinnicutt and Mixter: Actinomycosis Treated with Vaccines, Boston Med. and Surg. Jour., 1912, p. 90.

Mixter—two abdominal, two pulmonary and four cervicofacial. The pulmonary cases were advanced, and no gain was shown under treatment, but for the first few days a great increase in the granules in the sputum occurred. Of the two abdominal infections, one patient was cured and one died. Three of the jaw and neck patients recovered, and one had almost recovered at the time of writing. Kinnicutt and Mixter conclude that vaccines are useful in *local* infections, but are not of marked effect in deep-seated or advanced cases.

The widely differing preparations used, the relatively few cases recorded, and especially the lack of diagnosis of early infections, leaves but a slight basis for estimating either the curability of the disease or the best methods of treatment. There is no doubt, however, that early recognition and prompt treatment, either by iodin or vaccine, would help more of the patients to recovery than are at present cured.

CONCLUSIONS

1. Streptotrichosis is more common than generally supposed and frequently not differentiated from other infections; especially is it confused with tuberculosis. Clinical signs are not characteristic enough to determine the diagnosis accurately.

2. Discharges—sputum, pus, etc.—should be carefully examined fresh in as large quantities as possible, and smears stained with carbolfuchsin and Gram stains. The amount of decolorization in the carbolfuchsin preparations should be varied so as to differentiate the less acid-fast types.

3. Streptotrichins (glycerinated bouillon cultures, concentrated), made from the non-acid-fast mycelial and partly acid-fast bacillary organisms, give definite skin reactions on persons with frank infections, but do not show on controls (persons presumably non-infected) including cases of recognized tuberculosis. This either shows the infection to be less general than that due to the tubercle bacillus or else the reaction to be a less long-lasting one.

4. Streptotrichosis and tuberculosis may exist in the same person at the same time, as evidenced by the appearance of both organisms in the sputum and both skin reactions.

5. Gland and bone infections with streptotriches may be demonstrated by skin reactions as well as lung infections. This still further broadens the field for confusion between the tuberculoses and streptotrichoses.

I wish to express my thanks to the physicians in charge of the Alameda County Society for the Study and Prevention of Tuberculosis of the University of California Hospital, and of the Alameda County Hospital for their courtesy in allowing me to use clinical material in these institutions.

A PRELIMINARY REPORT ON THE PRODUCTION, ACTION AND THERAPEUTIC EFFECTS OF LEUKOCYTIC EXTRACTS*

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Subsequent to the publication of an article by Hans Zinsser, George W. McCoy and C. W. Chapin[1] on The Protective Influence of Leukocytic Substances on Experimental Plague, one of us conceived the idea that if leukocytic substances could be obtained in sufficient quantities to be not only therapeutically practical, but commercially possible, a decided step forward would be made in the biological treatment of infectious diseases.

Prior to the publication of the above mentioned article, Hiss and Zinsser[2] demonstrated in an experimental way that leukocytic extracts could be prepared by injecting into the pleural cavities of rabbits 10 c.c. of a meat extract broth containing 3 per cent. starch and 5 per cent. aleuronat. At the end of twenty-four hours a copious, and very cellular exudate will have accumulated in the pleural cavities. This is obtained by killing the rabbits, and removing the exudate which is immediately centrifuged. The leukocytic sediment is diluted with twenty volumes of sterile distilled water to one volume of sediment, and autodigestion is permitted to take place in the incubator, following which the extract is stored in a refrigerator until used.

In experimenting with animals, Hiss and Zinsser observed that pneumococcus, staphylococcus, streptococcus, meningococcus, typhoid, dysentery, and cholera infections in rabbits and guinea-pigs, were profoundly modified when injections of leukocytic extracts prepared as above described were administered intraperitoneally or subcutaneously during the course of the infection. In many cases, animals were saved by these substances from infections which proved rapidly fatal in untreated control animals, even when the protective injections were made as late as twenty-four hours after intravenous infections.

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* From the Research Department of the Western Laboratories, Oakland, Cal.
* Read before the Medical Research Society of the East Bay Counties, Oakland, Cal., April 4, 1914.
* Submitted for publication, April 30, 1914.

1. Zinsser, Hans, McCoy, Geo. W., and Chapin, C. W.: The Protective Influence of Leukocytic Substances on Experimental Plague, Jour. Med. Research, 1911, xxiv, No. 3.

2. Hiss, P. H., and Zinsser, Hans: Jour. Med. Research, 1908, xiv, No. 3; 1909, xv, No. 3.

In applying this method of treatment by subcutaneous injections to infections in man, Hiss and Zinsser observed distinctly beneficial results in cases of epidemic cerebrospinal meningitis, lobar pneumonia, staphylococcus infections, and erysipelas.

Hiss and Zinsser's idea was that injecting into infected subjects the substances composing the cells usually found in exudates in a diffusible form, such substances would, following absorption, relieve or stimulate the fatigued leukocytes.

Later, Zinsser, assisted by McCoy and Chapin, conducted a series of experiments on white rats with a view to ascertaining whether substances obtained from normal leukocytes would modify in any way the course of experimental infection with the bacillus pestis. As the results of their experiments these workers found that the use of emulsions of normal leukocytic substances obtained from rabbits exerted a distinctly protective influence, even when such substances were administered twenty-four hours after the inoculation of rabbits with virulent plague organisms.

While the results obtained by the above mentioned investigators were intensely interesting from a practical point of view their method of obtaining leukocytic substances must be considred as of little value, since the injection of a protein substance into the pleural cavities of rabbits yields such a small amount of leukocytic substance as to rob it of any value therapeutically.

Furthermore, the leukocytes so obtained are, strictly speaking, inflammatory leukocytes, which have probably given up much of their potential qualities, or in other words, have become fatigued or exhausted with the task of caring for the foreign protein, the injection of which caused them to migrate into the pleural cavity.

For the purpose of throwing some light on the nature of bactericidal substances in leukocytic extracts, Wilfred H. Manwaring[3] conducted a series of experiments. Manwaring started out with the assumption that there exists powerful bacteriolytic substances within the cytoplasm of certain body cells, and that this fact is amply demonstrated in the automatic sterilization of old abscess cavities and the sterilizing of the pneumonic lung, which is assumed to be due to the liberation of endolysins, the result of cellular disintegration. His work was done with a view of extending the present knowledge of these lytic substances, and to determine the approximate chemical nature of the bacteriolytic substances that might be extracted from leukocytes.

3. Manwaring, Wilfred H.: The Nature of the Bactericidal Substances in Leukocytic Extract, Jour. Exper. Med., 1912, xvi, No. 3.

In obtaining leukocytes for these experiments, Manwaring at first followed the practice of injecting a 5 per cent. suspension of aleuronat into the pleural cavities of rabbits, but he found that he was decidedly handicapped in his study of the nature of the extracts so obtained on account of the small amount of substance available. By injecting aleuronat into the pleural cavity of a rabbit, one seldom secured more than a single cubic centimeter of leukocytes. In order to overcome this obstacle, he selected the horse for the continuation of his work. His treatment consisted in injecting into the pleural cavity of a horse, from 300 to 500 c.c. of a 5 per cent. aleuronat in 2 per cent. starch-paste solution, the starch being added to aid in holding the aleuronat in suspension. Following such an injection there is usually found from 1 to 3 liters of pleural exudate daily for the first week; the daily yield then decreases, usually ceasing entirely about the fifteenth day. The exudate contains about 5 per cent. of leukocytes.

Manwaring, while not being able to arrive at a final conclusion as to the nature of the bactericidal substances in leukocytic extracts, did reach the logical conclusion, as the results of his investigations, that the bactericidal substance present is probably an enzyme.

We could, did circumstances permit, go on and recount the results obtained by other investigators, showing that leukocytes do contain intracellular substances which possess proteolytic properties, but from a study of the question from a therapeutic standpoint, the results obtained by later investigators, and our own personal work, indicate that the direct bactericidal action brought about by the parenteral injection of leukocytic substances does not influence to a marked degree the progress of an infectious disease. While able to demonstrate *in vitro* bactericidal properties in leukocytic extracts produced by the method devised by us, it was long ago concluded that such bactericidal powers were not sufficient to account for the profound changes actually produced in animals suffering from infectious diseases while undergoing a course of treatment by the parenteral injection of leukocytic substances. For the reason of these profound changes wo must look elsewhere than to the direct lytic action on the invading bacteria.

It is a well-established fact that the parenteral injection of protein substances increases the leukocytic count, and even the injection of foreign vegetable proteins such as nuclein produce, in many infections, marked beneficial effects. It would seem, however, in carrying out leukocytotherapy, that in order to produce a hyperleukocytosis without unnecessary strain on the organism, it is eminently more logical for the organism to care for parenteral injections of substances similar to its own elements than to any elements which are of an alien character.

It is also a well established fact that in the treatment of diseases, any method which will stimulate the leukocyte-producing organs to a more active function, thereby producing a leukocytosis, is and must of necessity be of positive benefit. The great problem before us then in this connection is to ascertain the simplest and most innocent means of accomplishing this end.

About the beginning of the year 1911 a series of experiments were instituted with a view of obtaining leukocytic substances in quantities that would render them not only therapeutically practical, but also commercially possible. The general plan adopted was to obtain leukocytes directly from the blood of normal animals, avoiding as far as possible any changes in their character during the process. It is unnecessary to go into detail as to the various methods employed to bring this about. Suffice it to say that after much experimental work, the following general technic was adopted:

TECHNIC

The blood is obtained under aseptic precautions from the jugular vein of the horse, dog, or other domestic animal; is allowed to flow into sterile flasks containing a sufficient quantity of 1 per cent. sodium citrate solution to prevent coagulation. When the blood arrives at the laboratory, sodium citrate solution is added in varying amounts, depending on the species of animal from which the blood was obtained; to this is added five-tenths of 1 per cent. acetic acid, the necessary amount of which also depends on the character of blood being treated. The mixture is then centrifuged in a high powered centrifuge and the supernatant fluid is discarded. The sediment, which is very rich in leukocytes, is washed several times with physiological salt solution, ground with quartz sand and neutralized. To the resulting sediment distilled water is added in the proportion of about four volumes of water to one of sediment. This mixture is exposed to a temperature of 58 C. for one hour, after which it is placed in the incubator until digestion of the leukocytes is complete. It is finally centrifuged, the supernatant fluid decanted and sufficient trikresol added for preservative purposes. Each lot prepared as described is carefully examined bacteriologically and its ability to produce certain definite blood changes is determined by animal inoculation.

While a great many experiments have been carried out, not only on different animals, but also on human beings, for the purpose of shedding some light on the action brought about by the parenteral injection of leukocytic extracts, it is obviously unnecessary and impracticable to submit the great mass of data so obtained at this time. We wish, however, to present the results of a few experiments which will suffice to demonstrate some of the blood changes that follow injections of the extracts.

EXPERIMENTS

Experiment 1.—For the purpose of ascertaining the different effects, if any, following parenteral injections of leukocytic substances obtained from various animals into a series of animals of the same species, with a view of determining

whether the extracts of a different species will give rise to the same changes as leukocytic extracts from the same species, the following experiment was instituted:

Four guinea-pigs of equal weight were injected as follows: Pig 1 received 1 c.c. of horse leukocytic extract; Pig 2, 1 c.c. of dog leukocytic extract; Pig 3, 1 c.c. of guinea-pig leukocytic extract, and Pig 4, 2.5 c.c. nuclein (P. D.).

Chart 1 shows their total leukocytic curves and the total polymorphonuclear counts.

It will be observed that the injection of leukocytic extracts obtained from three different sources as well as the nuclein produced a marked leukocytosis. The leukocytic substances, with the exception of the guinea-pig extract, produced a greater reaction than the nuclein.

The dog extract produced the most marked reaction, the total leukocytic increase amounting to about 300 per cent. The differential

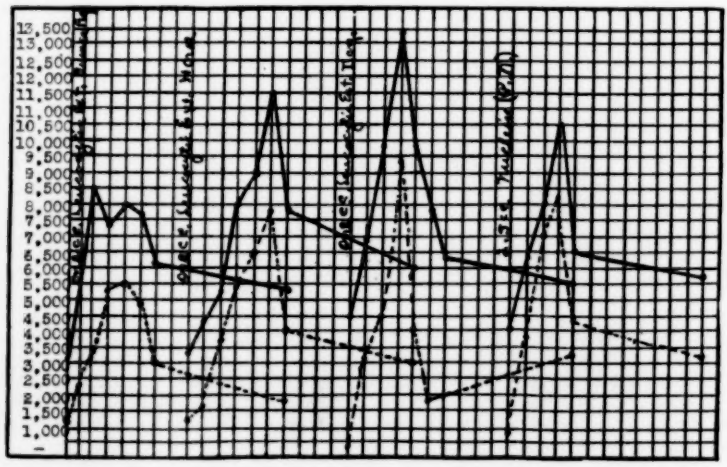

Chart 1.—Guinea-pig series. Each square represents a one and one-half hour interval. In this and following charts the solid line indicates total white count; line of short dashes, total polynuclear count; dotted line, total neutrophil count; dash and dot line (—.—.—.—.), total eosinophil count.

counts showed that the dog leukocytic extract caused the most profound changes, the neutrophil increase amounting to 350 per cent., and the eosinophil count increasing from 3 to 14 per cent. The large mononuclears showed, in many cases, bundles of chromatin in their protoplasm. The nuclei of these cells were crowded to one side with a loose net-work of chromatin in the protoplasm on the opposite side. These cells appeared about one and one-half hours after the injection; gradually increasing in number for about six hours, when about one-third of the cells showed this change. In the case of the guinea-pig injected with guinea-pig leukocytic extracts, the polymorphonuclear

leukocytes showed practically no granules and no red staining before the injection was made. A few red granules were noticed at the second count and at the third count, with but few exceptions, all the neutrophils showed distinct red granules in their protoplasm. These granules decreased in size and number until they had practically disappeared at the twenty-fourth hour after injection.

In the guinea-pig injected with horse leukocytic extract, the polymorphonuclear leukocytes showed no red granules before injection, but at the second count the red granules were at their height, disappearing at the third count, a few reappearing on the following day.

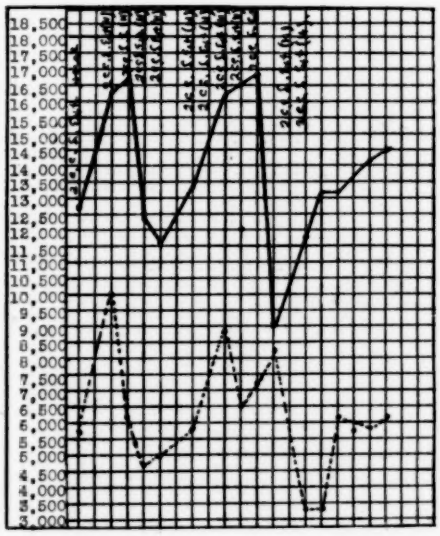

Chart 2.—Rabbit 1. Each square represents twenty-four-hour interval.

No counts could be made from the nuclein-treated pig until four and one-half hours after the injection, due to the fact that the blood clotted so rapidly. At this time a few red granules were observed in a few of the cells, but this phenomenon was not nearly so marked as in the animals injected with the leukocytic extracts.

Experiment 2.—Chart 2 shows the daily blood picture of Rabbit 1, which received twelve subcutaneous injections of 2 c.c. horse leukocytic extract at twenty-four and forty-eight-hour intervals. Blood-counts were taken daily, commencing at the time of the first injection, and continued for fifteen days. Smears made at the time of the first injection showed the protoplasm of the neutrophils, with a few exceptions, to be free from granules, the granules in these exceptions being very fine and taking a distinct red stain. At no time

after the injections of the extract were the granules as fine, nor as few in number as before. Practically all the polymorphonuclear cells contained large, distinct, red granules; these granules had not decreased in number seventy-two hours after the last injection, at which time observations ceased.

It will be observed that marked total leukocytic curves occurred throughout the experiment. Differential counts showed that the increase in the eosinophil count and decrease of the neutrophil count, and the increase of the neutrophil count and the decrease of the

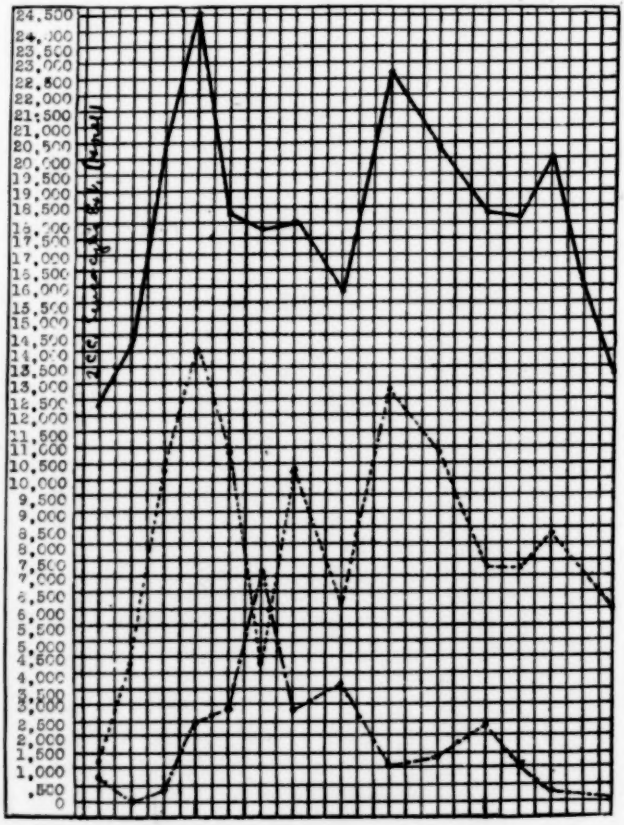

Chart 3.—Rabbit 2. Each square represents a one-hour-interval.

eosinophils kept pace with the rise and fall of the total leukocytic count. When the leukocytes took an upward curve the eosinophils also increased, reaching at one time a height of 45 per cent.

Experiment 3.—Rabbit 2 was given a single injection of 2 c.c. horse leukocytic extract. Blood-counts, as shown in Chart 3, were taken at two-hour intervals for thirty-six hours. The neutrophils showed much the same normal

picture as in Rabbit 1. The red granules began to appear four hours after injection, and increased to a maximum in size and number between the fourth and sixth hours. These granules did not disappear during the time of the experiment, but decreased in size and number from the tenth hour on. The total count showed a drop during the first two hours, and then took an upward curve reaching the maximum height of 100 per cent. above the normal about the sixth hour. The differential counts showed an increase and decrease of the eosinophils and neutrophils coincident with the curves formed by the total leukocytic counts. The total neutrophil count at its maximum reached a point of 700 per cent. above the normal and the eosinophil total count 1,100 per cent. above the normal.

Experiment 4.—With a view of demonstrating the effects of leukocytic extracts on a human being the following experiment was carried out:

Chart 4 shows the blood pictures of a man subsequent to a subcutaneous injection of 2 c.c. of dog leukocytic extract.

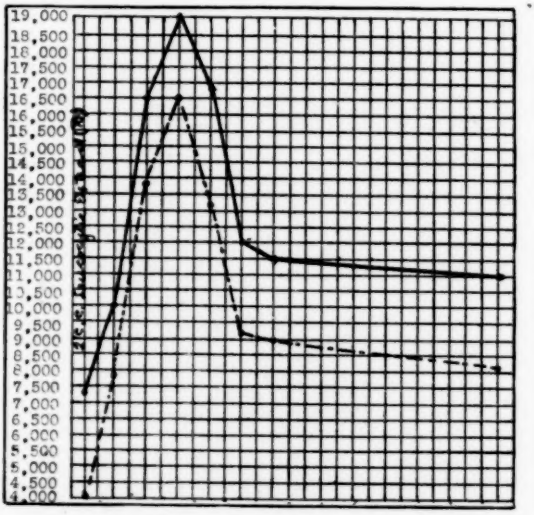

Chart 4.—Normal man. Each square represents a one-hour-interval.

A white and differential count was made just prior to the injection and subsequent counts were made at two-hour intervals for a period of twelve hours. A final count was made twenty-six hours after the injection.

The curve showed a continual increase beginning two hours after the leukocytic extract was administered, reaching its maximum at about the sixth hour. At this point the total leukocytic count was slightly over 150 per cent. above the normal and the polymorphonuclears had increased to about 500 per cent. From the sixth to the twelfth hours there was a gradual decrease both of the total leukocytic and polymorphonuclear counts. Twenty-six hours after the injection, the count had not changed from that found at the twelfth hour. At this time the total leukocytic count was about 50 per cent. above the normal, with a polymorphonuclear count a little over 100 per cent. above the normal.

The therapeutic application of leukocytic extracts to humans and animals has been followed out during the past three years. Various infections have been treated, in some types, more especially acute

infections, with most gratifying results, while in others, especially old chronic infections, the beneficial results were not so marked. Some of the diseases of human beings to which the treatment has been applied, and which have yielded the most pleasing results, are pneumonia, puerperal sepsis, erysipelas, empyema, bacteremia, acute pyemia, gonococcus infection, rheumatism, etc.

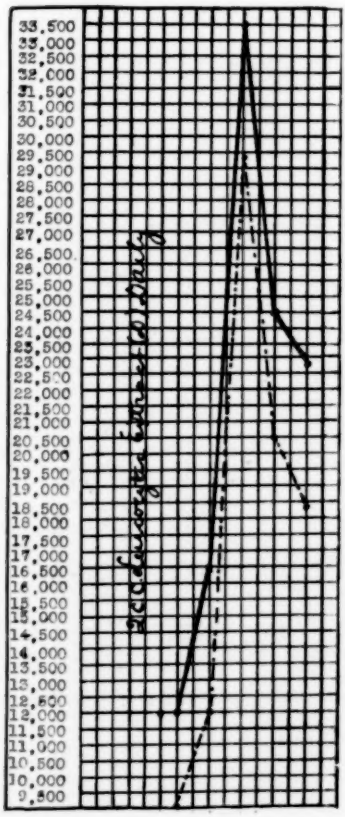

Chart 5.—Lobar pneumonia (left lower lobe). Each square represents a twenty-four-hour interval.

The following are a few of the records selected from a number of human cases treated with leukocytic extracts:

CASE REPORTS

Case 1 (Chart 5. Lobar Pneumonia).—Miss M., a Portugese school-girl aged 16, was first seen at 2 p. m., March 10, at which time she gave a history of a severe chill twenty-four hours previously. This was followed by fever,

headache, nausea, vomiting, and pain in the left chest. Examination showed a temperature of 103.6 F., pulse 112, respiration 36, white blood-count 12,000, polymorphonuclear cells 70 per cent. with a dull area over the lower lobe of the left lung. Two cubic centimeters of leukocytic extract was given once daily for eight days. Following the first dose of extract the white blood-count rose to 33,400 with 88 per cent. polymorphonuclear cells, and the total count remained above 23,000 during the remainder of her illness. After the third dose the temperature came down by lysis, reaching normal on the eleventh day of the disease.

CASE 2 (Chart 6. Cellulitis).—The patient, a young man, when first seen complained of a swelling, redness, and pain in the right arm and adjacent chest wall. The constitutional symptoms of nausea, vomiting and headache were severe, the temperature was 103 F. and there was no tendency to localization or pus formation. At the time of admission to the hospital the white blood-count was 25,000; unfortunately no differential was made.

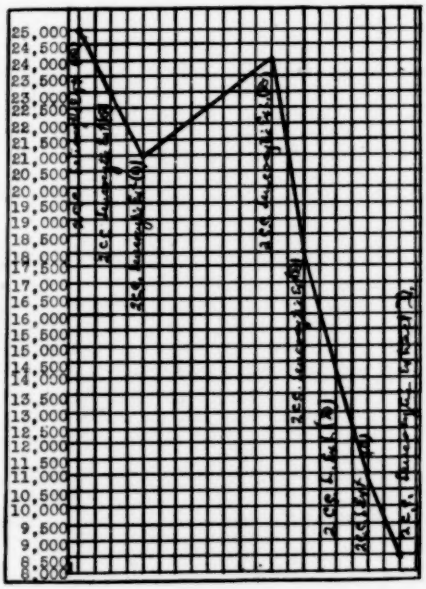

Chart 6.—Cellulitis. Each square represents a twenty-four-hour interval.

The treatment at this time consisted in the subcutaneous injection of 2 c.c. of leukocytic extract once daily, with the exception of the second dose, which followed the first after a period of twelve hours. Both the local and general symptoms were very much improved after the second dose and on the third day the temperature was normal, the patient felt well except for a slight tenderness in the affected part, the skin was normal in color, but there still remained some swelling. On account of the improved condition, it was thought best to discontinue the use of the extract at this time. That the treatment should have been continued longer was evidenced by the fact that on the seventh day, which was the fourth day without treatment, all the local and general symptoms returned, following a sudden and severe chill. The temperature again

reached 103 F. and the total leukocyte count 24,000. Two cubic centimeters leukocytic extract were again given once daily for the next six days. The general symptoms as before, quickly improved, and following the second dose the infection became localized and an abscess formed which was drained surgically. Healing was rapid and on the twelfth day after admission the patient left the hospital well. The chart accompanying this record is most interesting. It will be noticed that the high blood-count on admission decreased after the first doses of the extract, and increased when the extract was discontinued, to again decrease as soon as the treatment with the extract was resumed. As no blood-counts were made on the fourth, fifth and sixth days, the lowest blood-count reached following the first doses of extract is not known.

Case 3 (Puerperal Sepsis).—On the third day following confinement Mrs. G. developed a temperature of 104 F. with nausea, vomiting and intense headache. On the following day she had several slight chills, with temperature continuing high, and accompanied by some delirium and later stupor. The patient was then removed to the hospital and 2 c.c. leukocytic extract given daily. The following day she was brighter, said she felt better, temperature dropped to 102 F. There was no further delirium, improvement continued, and the temperature reached normal four days after the administration of the extract. A blood culture made at the time this patient entered the hospital showed the presence of the *Staphylococcus aureus*.

Case 4 (Empyema).—Mr. B. injured by a fall from a telephone pole Oct. 17, 1913, was removed to the hospital, Oct. 22, when an abdominal operation was performed for the relief of a general peritonitis. Later an empyema developed which was operated on November 20. The temperature, which had ranged from 102 to 104 F. up to this time, fell to normal following the removal of the pus from the pleural cavity, but the pyothorax quickly returned and the temperature again rose, necessitating a second aspiration. A glycerin-formaldehyd solution was introduced after each aspiration. Up to December 13, when the daily subcutaneous injections of leukocytic extract were commenced, the temperature had ranged between 101 and 102 F., except for brief intervals following drainage. From this time on, however, the temperature fell slowly, reaching the normal on December 28, when the patient reported feeling well and on December 30 left the hospital with no signs of empyema.

Case 5 (Facial Erysipelas).—Mr. S., aged 60, when first seen presented an erysipelas of three days' duration involving the entire face, neck and both ears. His temperature at this time was 105 F. The treatment consisted of daily subcutaneous injections of 2 c.c. leukocytic extract. After the second dose the inflammation ceased to spread, the affected area became lighter in color, and the temperature fell to normal. On the twelfth day after the onset the patient returned to his work, while in previous attacks he had been confined to his bed for from eighteen to twenty-one days.

Some interesting data concerning "Clinical Studies on the Curative Action of Leukocytic Extracts in Infective Processes" by Hiss and Dwyer,[4] are worthy of note in this connection. In this article the results obtained by the use of leukocytic extracts on a number of cases of erysipelas are tabulated.

Total number of cases treated, 148.
Recovered, 143; mortality = 3.38 per cent.
General average duration of treatment, 3.18 days.
Number of recoveries of patients under 1 year old, 8 out of 12.
Percentage of recoveries of patients under 1 year old, 66.6 per cent.
Mortality among patients over one year, 0.73 per cent.

4. Hiss and Dwyer: Clinical Studies on the Curative Action of Leukocytic Extracts in Infective Processes, New York Med. Rec., 1913, lxxxiv, No. 11.

These authors also refer to the results obtained by Dr. Adrian Lambert who treated fifty cases of erysipelas at the Bellevue Hospital and obtained 50 per cent. recoveries of babies under one year. Dr. Lambert concluded from his experience that leukocytic extracts will abort infections which are treated within twenty-four hours. Older cases are ameliorated and may abruptly terminate. Toxic symptoms, delirium, headache, nausea, vomiting and pain are relieved. Rash does not disappear immediately, but is apt to localize.

It is fully realized that these therapeutic agents are at present undergoing the "acid test" and it is difficult to prevail on the therapeutist to give them a trial, unless as a last resort. Hence the list of infections for which we can conscientiously recommend the treatment is more limited than it should be. It is believed, however, from our experience in the treatment of animal diseases, that leukocytic extracts have a wide range of therapeutic application and that their use is warranted in all acute infections at least.

In animals the treatment has been applied to a great variety of local and general infections; such diseases of the horse as pneumonia, purpura, influenza, laminitis, strangles, and many local infections have yielded more rapidly to the use of the extracts than to any other treatment heretofore applied. In such diseases of the dog and cat as pneumonia, so-called distemper, and the very common bowel infections in these small pet animals, the results have been most gratifying.

In some of the above mentioned diseases in animals the agent seems to act as a specific, if we may be permitted to use the term. Apparently hopeless infections, even those in which the mortality is ordinarily very high, make good recoveries following daily injections of the extracts.

In such cases as purpura in the horse and distemper in the dog, where the treatment is commenced before the animals have lost their reactive powers, about the third day following the daily injections of the extracts rapid improvement in the clinical manifestations are observed, and this improvement continues until recovery is complete. provided the treatment is kept up until immunity has been well established.

It has been invariably observed that the first and second injections of leukocytic extracts have the tendency slightly to aggravate the toxic symptoms, even to the extent of producing slight uneasiness on the part of the affected animal, but these conditions are soon followed by improvement. These observations would seem to demonstrate that the initial effect of the extract is to increase intoxication by the liberation of toxic elements, which, providing the individual is capable of react-

ing, stimulate the machinery of immunization and the consequent production of immune elements in sufficient quantities to control or overcome the infection.

We fully realize that there are a great many problems incidental to the production, nature, and effects of leukocytic substances that require solution, but we believe that what has thus far been accomplished may prove to be of some assistance in future study and investigation. We also believe that the facts already obtained warrant the following conclusions:

CONCLUSIONS

1. Leukocytic substances possessing positive therapeutic value may be procured from leukocytes obtained directly from the blood of normal animals.

2. Leukocytic substances may be obtained directly from the blood of normal animals in quantities which render them not only therapeutically practical but commercially possible, conditions that can hardly be claimed for leukocytic extracts prepared from inflammatory leukocytes.

3. The parenteral injection of leukocytic substances causes profound changes in the blood, producing a marked leukocytosis and marked changes in the individual leukocytes. As to the significance of these phenomena no opinion can be offered at this time.

4. Leukocytic extracts have not sufficient direct bactericidal powers to account for the profound changes produced following their parenteral introduction into an animal suffering from an infectious disease, and the beneficial results obtained are probably due to the fact that the extracts furnish elements which stimulate the fatigued or exhausted leukocytes, enabling them to carry on their function of phagocytosis and produce the changes incidental thereto.

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A COMPARISON OF BANG'S MICROMETHOD FOR DETERMINING BLOOD SUGAR WITH BERTRAND'S METHOD *

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It is recognized that the constituents of the blood are of as great a clinical significance as the constituents of the products of excretion, but heretofore blood analysis has not been undertaken as a matter of routine in the clinic, on account of the technical difficulties in the methods employed or because of the large amounts of blood required. Folin and Denis,[1] however, have recently described relatively simple and accurate ways for estimating the non-protein nitrogen, urea, ammonia, and uric acid of the blood, and Marshall[2] has published a method for determining blood urea. These various tests are proving of great value, because clinicians are now able to estimate the nitrogen-containing elements of blood as part of the ordinary study of different types of disease.

Since Claude Bernard[3] recognized that glucose could be found in the circulating blood, a large literature on blood-sugar has accumulated. Two methods for blood-sugar determinations have been generally used. After the proteins from the blood have been removed, the sugar in the filtrate has been quantitated by polarization, or by one of the metallic reduction tests.

The blood proteins have been taken out by numerous reagents. Bernard and Röhmann[4] chose sodium sulphate, Abeles[5] selected an alcoholic solution of zinc acetate or chlorid, precipitating the zinc by sodium carbonate and washing the sugar into the filtrate. According to Schenck,[6] the proteins could be precipitated with mercuric chlorid, which was removed by hydrogen sulphid. MacLeod[7] concluded in later experiments, however, that certain substances in addition to the proteins were brought down by the mercury and that therefore the results so obtained were not absolutely accurate. He advised the use of

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* From the Medical Clinic of the Peter Bent Brigham Hospital.
* Submitted for publication, March 18, 1914.

1. Folin and Denis: Jour. Biol. Chem., 1912, xi, 527.
2. Marshall: Jour. Biol. Chem., 1913, xv, 487.
3. Bernard: Leçons de physiologie, 1855, Baillière, Paris.
4. Röhmann: Centralbl. f. Physiol., 1890, iv, 12.
5. Abeles: Ztschr. f. physiol. Chem., 1891, xv, 495.
6. Schenck: Arch. f. d. ges. Physiol. (Pfluger's), 1894, lv, 203.
7. MacLeod: Jour. Biol. Chem., 1909, v, 443.

phosphotungstic acid as described by Reid[8] to obtain the sugar solution. A disadvantage of all these methods lay in the fact that a certain amount of sugar was held back from the filtrate during precipitation. As a result the precipitates had to be washed sugar-free to obtain accurate estimations and this procedure required a large bulk of fluid, and considerable time was occupied in later concentrating this fluid.

Edie and Spence[9] attempted to obviate this by dialysis of the sugar from the blood into saline solution. Michaelis and Rona[10] introduced the use of colloidal iron in the presence of an electrolyte. They were able to precipitate the blood proteins completely by these reagents without absorption of sugar and therefore they could use aliquot parts of the filtrate instead of washing the precipitate. Furthermore since the iron and salt were precipitated with the protein, no necessity existed for repeated filtering. Thus this method was more rapid and simple than the older ones. Bang,[11] however, has preferred alcohol and animal charcoal as a means of obtaining the sugar filtrate.

The polarization of blood-sugar solutions has been advocated by Michaelis and Rona and their pupils. The majority of workers on the various means of quantitative sugar analysis have used one of the reduction tests. These are all based on the same underlying principle. It is known that a cupric or mercuric salt is reduced quantitatively by sugar and boiling, and if the amount of copper or mercury involved in the reaction can be estimated, the total amount of sugar in a given solution can be determined. The various tests most generally used have been gravimetric, titrimetric or colorimetric.

Allihn devised a method, modified by Pflüger[12] in which a solution of copper sulphate was boiled with an unknown sugar solution. The reduced copper was filtered, ignited and weighed as pure copper. From this figure the amount of sugar present was estimated from especially prepared tables giving the value of varying amounts of sugar in terms of copper. This test or one slightly modified has been used by Bönniger,[13] Edie and Spence, Harley,[14] MacLeod and Reid. Bertrand,[15] instead of weighing the reduced copper, dissolved it in a solution of ferric sulphate and sulphuric acid. The amount of ferrous salt so formed was determined by titration against potassium permanganate

8. Reid: Jour. Physiol., 1896, xx, 316.
9. Edie and Spence: Biochem. Jour., 1907, ii, 103.
10. Michaelis and Rona: Biochem. Ztschr., 1908, vii, 329.
11. Bang: Ztschr. f. physiol. Chem., 1910, lxv, 497.
12. Pflüger: Arch. f. d. ges. Physiol. (Pfluger's), 1898, lxix, or 1902, xcvi, 1.
13. Bönniger: Deutsch. med. Wchnschr., 1908, xxxiv, 780.
14. Harley: Jour. Physiol., 1891, xii, 391.
15. Bertrand: Quoted from Grube, Abderhalden's Handbuch der biochemischen Arbeitsmethoden, 1910, ii, 181, Urban and Schwarzenberg, Berlin and Vienna.

standardized with ammonium oxalate. Knowing this, the amount of copper present in the reaction was found, and the amount of sugar in an unknown solution was estimated by tables giving different copper-sugar values.

Bernard used Fehling's method. An alkaline copper solution was made of such strength that it was perfectly reduced on boiling with a fixed amount of sugar. In making an analysis, a part of the sugar solution was added to the copper until no further reduction occurred and the ratio which this bore to the entire solution gave information from which it was possible to discover the total quantity of sugar present. The difficulty with the test lay in the fact that the end-point was uncertain. Pavy[16] modified the reaction by making a solution of ammoniacal copper which held the reduced copper in solution but lost its blue color after complete reduction occurred. A known amount of copper was reduced by a part of the unknown sugar solution, and from this titration the entire amount of sugar present could be estimated. Kumagawa and Suto[17] have obtained good results by its use.

Bang's[18] test is on somewhat the same principle. The reduced copper is held in solution by potassium rhodamate in the presence of carbonates. In performing the test an excess of copper solution is added to the sugar, and is later reduced to a colorless solution by hydroxylamin. The amount of sugar taking part in the reaction is estimated by tables prepared from known sugar solutions and the reagents.

Instead of using copper as a reduction test, Knapp[19] selected mercuric cyanid which is reduced into metallic mercury by glucose. A solution was made which could be reduced by a known amount of sugar, and titration was carried out until no mercury salt was left in solution. Röhmann, Schenck and Liefmann and Stern[20] used the method in their studies on blood-sugar, while Tachau[21] modified it to serve as a rapid test for clinical purposes.

Wacker[22] introduced colorimetric means for determining blood-sugar. The albumin in the blood was precipitated by iron alum and sodium carbonate and the amount of sugar in an aliquot part of the filtrate was found by a sensitive color reaction between the sugar and a solution of phenylhydrazin sulphacid in concentrated sodium hydrate.

16. Pavy: Pavy, Chem. News, 1880, ix, 98, Abs. Ztschr. f. anal. Chem., 1880, xix, 98.
17. Kumagawa and Suto: Salkowski's Test, Salkowski's Festschrift, 1904, p. 211, Hirschwald, Berlin.
18. Bang: Biochem. Ztschr., 1906, ii, 271.
19. Knapp: Quoted from Hammarsten. A text-book of physiological chemistry, 1893, 417, John Wiley & Sons, New York.
20. Liefmann and Stern: Biochem. Ztschr., 1906, i, 299.
21. Tachau: Deutsch. Arch. f. klin. Med., 1911, cii, 597.
22. Wacker: Ztschr. f. physiol. Chem., 1910, lxvii, 197.

Quantitative readings were obtained by comparison of the color of unknown solutions with that of known sugar solutions mixed with the reagents. Autenrieth and Tesdorpf[23] boiled sugar solutions with Bang's copper solution, diluted the mixture to a known amount and estimated the amount of reduced copper by colorimetry. The copper solution lost color in proportion to the amount of sugar present, so that Autenrieth and Tesdorpf were able to construct a color scale of sugar values which gave accurate readings. Forschbach and Severin[24] and Borchardt and Bennigson[25] used this method satisfactorily. Reicher and Stein[26] depended on Molisch's reaction as a quantitative test. Tablets of alphanaphthol and sulphuric acid were added to a sugar solution from which the albumin was precipitated and the color changes were compared with tubes containing similar amounts of reagent and increasing amounts of sugar. The readings obtained were higher than by the other methods, which made Forschbach and Severin believe that the test was not sufficiently delicate for general use.

From this review it is seen that there are a variety of ways for estimating blood sugar, none of which are free from criticism. Difficulties have been encountered not only in precipitating the proteins from the blood but also in getting the sugar values. Constant readings, however, which have been valuable in comparative studies over long intervals of time, have been obtained by using any one method. The objections to the various tests from the point of view of the clinician have been the large amounts of blood required to conduct the analyses, the length of time for their completion, and the difficulty of technic.

Any test, to be of use in clinical studies on blood analysis, must require small amounts of blood, so that it can be repeated at will, must be accurate enough for comparative readings, and must be so simple that it can be performed by individuals without specialized training in chemical technic. Bang[27] has described a method for blood-sugar analysis which fulfills these requirements. Thannhauser and Pfitzer,[28] Götzky,[29] Bing and Windelöw,[30] Bing and Jacobsen[31] and Hirsch and Reinbach[32] have confirmed the value of this test, but have

23. Autenrieth and Tesdorpf: München. med. Wchnschr., 1910, lvii, 1780.
24. Forschbach and Severin: Zentralbl. f. d. ges. Physiol. u. Path. d. Stoffwechs., 1911, n. f. vi, 54, 177, 665.
25. Borchardt and Bennigson: München. med. Wchnschr., 1913, lx, 2275.
26. Reicher and Stein: Biochem. Ztschr., 1911, xxxvii, 321.
27. Bang: Der Blutzucker, 1913, J. F. Bergmann, Wiesbaden.
28. Thannhauser and Pfitzer: München. med. Wchnschr., 1913, lx, 2155.
29. Götzky: Ztschr. f. Kinderh., 1913, Orig., ix, 64.
30. Bing and Windelöw: Ztschr. f. Kinderh., 1913, Orig., ix, 64.
31. Bing, L., and Jakobsen, B.: Ugeskr. f. Laeger, 1913, lxxv, 1627; Abst. Jour. Am. Med. Assn., 1913, lxi, 1946.
32. Hirsch and Reinbach: Ztschr. f. physiol. Chem., 1913, lxxxvii, 122.

compared it with other methods in a comparatively small number of cases. In this country no systematic use of Bang's test has been reported.

This paper gives the results of a series of blood-sugar determinations made by Bang's micromethod and controlled by Bertrand's method to determine if the micromethod could be used with sufficient accuracy and sufficiently constant readings for clinical use. The technic of the two methods as used by the observer is given and the results are tabulated.

Twenty-five c.c. of blood were withdrawn from a vein according to usual technic into a small flask containing a few crystals of potassium oxalate to prevent clotting or were defibrinated by shaking. This blood was used for subsequent analysis either immediately, or after standing for a few hours to induce glycolysis. Each analysis by the two methods, however, was made at the same time. Two or three readings were made by the micromethod, and one by the Bertrand. Unfortunately enough blood for double determinations throughout was not obtained.

BERTRAND METHOD

The proteins were precipitated according to Rona and Michaelis. From 15 to 20 c.c. of blood were pipetted into a flask and were laked with 100 c.c. of distilled water; 110 c.c. of dialyzed iron solution[33] were added and 10 c.c. of saturated sodium sulphate solution. The mixture was diluted to 350 c.c. with distilled water, and was shaken for five or ten minutes. On filtering, a clear filtrate was usually obtained. In a few cases, re-filtration was necessary after the addition of a few additional cubic centimeters of the iron solution. An aliquot part of the filtrate, representing from 10 to 15 c.c. of blood was measured off, made acid with acetic acid and evaporated to 10 c.c. bulk on the water-bath. The mixture was then washed carefully into a 200 c.c. Erlenmeyer flask and the sugar estimation was conducted according to Bertrand's technic described by Grube.[34]

The sugar solution was boiled with Bertrand's two solutions for three minutes. On cooling the cuprous oxid was filtered and washed on a Gooch crucible, and was re-dissolved in a solution of ferric sulphate and sulphuric acid. The total copper present was determined by titration with potassium permanganate, and from the amount present the sugar was calculated by Bertrand's tables. From beginning to

33. The solution used was Merck's dialyzed iron (liquid 5 per cent. Fe_2O_3), the estimation was conducted according to Bertrand's technic described by Grube.[34]

34. Grube: Abderhalden's Handbuch der Biochemischen Arbeitsmethoden, 1910, ii, 181, Urban and Schwarzenberg, Berlin and Vienna.

end, the entire test took from three to four hours. In the few cases in which double determinations were made, the results checked with accuracy, so that on the whole the method appeared to give constant readings and was perfectly satisfactory as a control.

BANG'S MICROMETHOD

The tests were made according to Bang's original method as modified by Thannhauser and Pfitzer. The test depends on the assumption that copper is reduced by sugar without regard for the amounts present. In other words, a sugar solution from 100 mg. of blood will reduce as much copper proportionately as a solution from 100 c.c. of blood. If the microscopic amount of copper involved in the reaction can be determined the results will be as accurate as if larger amounts of the metal are used. Accordingly an albumin-free sugar solution is made from a small amount of blood and is boiled with copper solution. The cuprous oxid which is formed is held in solution by the method which Bang has described previously, and is reoxidized by iodin in the presence of potassium carbonate. Cupric carbonate and potassium bicarbonate are formed while potassium iodid is set free and can be tested for by starch as an indicator. The reaction is quantitative and extremely delicate. The technic for the test is given in detail.

The copper solution is made by dissolving 160 gm. of potassium bicarbonate in from 600 to 700 c.c. of distilled water, 100 gm. of potassium carbonate are added, 66 gm. of potassium chlorid and 100 c.c. of a 4.4 per cent. copper sulphate solution ($CuSO_4 + 5H_2O$). When everything is dissolved the mixture is diluted to one liter, and is ready for use after it is allowed to stand for twenty-four hours. In making an analysis this solution is diluted with a 20 per cent. potassium chlorid solution made from 150 c.c. of saturated solution plus 70 c.c. of water. Titration is made with one-hundredth normal iodin solution prepared from 1 c.c. of 2 per cent. potassium iodate solution, 2 gm. of potassium iodid and 5 c.c. of one-tenth normal hydrochloric acid, the mixture being diluted to 50 c.c. This solution is unstable and must be renewed every three or four days.

Two or three drops of blood are placed on a small piece of thick blotting-paper, which previously has been boiled in dilute acetic acid and water to remove traces of albumin and sugar. The blotting-paper should be cut in oblongs 10 by 20 mm. and weighed before and after the blood is added. In this way the weight of blood to within 1 mg. is found. In these experiments small weighing flasks were used to hold the blood and paper. After the blood is weighed, 7 c.c. of boiling hot potassium chlorid solution is added and acidified with 2 or 3

drops of 40 per cent. acetic acid. The albumin in the blood precipitates on the blotter, while the sugar diffuses through into the solution.

After half an hour's cooling the sugar solution is transferred to a 50 c.c. Jena flask with a straight neck and is washed in by 4 c.c. of the potassium chlorid solution. In this way all the sugar in the blood is removed from the weighing flask and is ready for titration.

Three c.c. of the copper solution are now added and the mixture is boiled over a micro-burner. The heating is an important factor in the test. The solution must be warmed so that it begins to boil in between one minute and fifteen seconds and one minute and a half, and must be boiled for exactly two minutes, since the copper solution can be reduced by longer or more vigorous boiling and will give improper readings. At the end of this time the flask is immersed in cold water and titrated.

A second source of error in the determination is air oxidation of the reduced copper. During boiling this is discounted. But after cooling, air must be kept out of the solution by manipulating it under carbon dioxid gas. For this purpose Bang introduced into the neck of the flask a hooked glass tube which was connected to a carbon dioxid tank. The gas was turned on as soon as the flask was put into cold water and in this way air oxidation was prevented. Thannhauser and Pfitzer used small flasks with side-arms which were easier to handle. Similar flasks were used in these experiments.

After the two minutes' boiling, the gas is introduced and the flask is immediately cooled. This can be done in less than three seconds. A few drops of 1 or 2 per cent. soluble starch solution are now added as an indicator. The titration is made from a 1 or 2 c.c. differential pipet reading in hundredths of a cubic centimeter, and the iodin solution is added until the light blue color of the copper solution takes on the deep blue color of the starch and iodid.

Bang has tested the method carefully with known sugar solutions. The amount of iodin used by increasing amounts of sugar is so nearly constant that he has been able to make a formula for determining the amount of sugar present. The amount of iodin used in titration is divided by 2.2. This result minus 0.01 will give the milligrams of sugar present in the solution. In these experiments a special table was constructed, however, which showed the iodin readings for varying amounts of sugar from which the blood sugar readings per 100 c.c. of blood were calculated.

The entire test takes less than three-quarters of an hour, requires so little blood that it can be repeated on the same individual at will, and is very easy to perform after short practice.

The results of forty-two blood examinations are given in the accompanying table. The Bang figures represent the average of duplicate readings unless otherwise noted. All but one or two read within 0.02 of a gram of each other per 100 c.c. of blood. The amounts of blood used, varied between 50 and 120 mg., although most constant readings were obtained when from 80 to 100 mg. were taken.

RESULTS OF FORTY-TWO BLOOD EXAMINATIONS

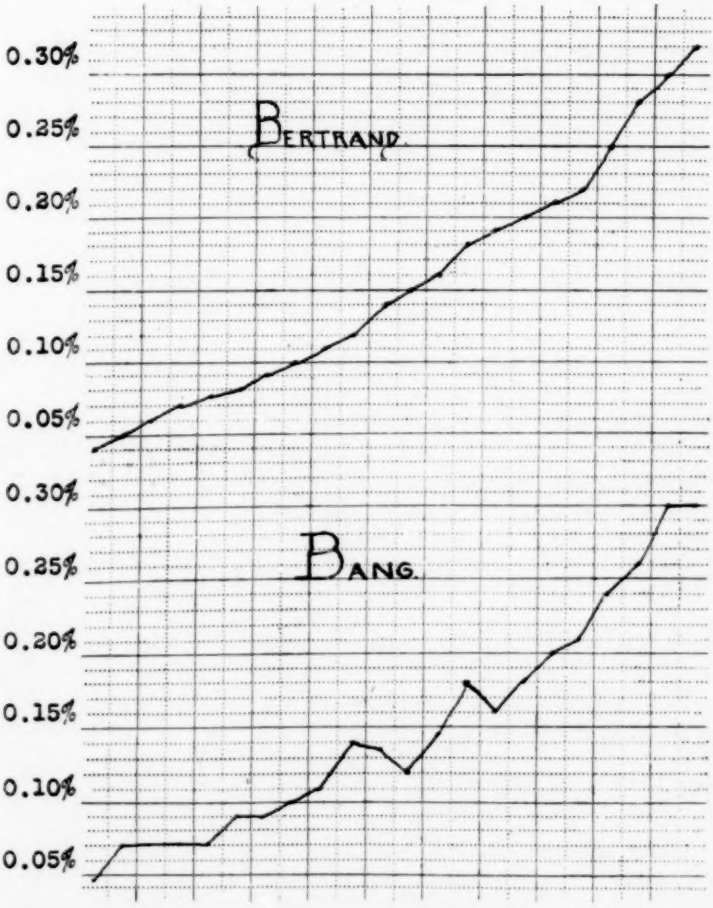
Blood Number	Methods		Blood Number	Methods	
	Bertrand* Per Cent.	Bang† Per Cent.		Bertrand* Per Cent.	Bang† Per Cent.
1	0.04	0.06	22	0.11	0.11
2	0.04	0.03	23	0.12	0.14*
3	0.05	0.06‡	24	0.14	0.12
4	0.05	0.085	25	0.14	0.15
5	0.06	0.08	26	0.15	0.12
6	0.06	0.075	27	0.16	0.145
7	0.06	0.07	28	0.16	0.15
8	0.06	0.05	29	0.18	0.17*
9	0.07	0.055	30	0.18	0.205
10	0.07	0.07	31	0.18	0.17
11	0.07	0.09	32	0.19	0.16
12	0.075	0.08	33	0.20	0.17
13	0.075	0.065	34	0.20	0.195
14	0.08	0.075	35	0.21	0.20
15	0.08	0.12	36	0.21	0.20
16	0.08	0.095	37	0.22	0.21
17	0.09	0.085	38	0.25	0.245
18	0.09	0.105	39	0.28	0.26
19	0.10	0.085	40	0.30	0.295
20	0.10	0.10	41	0.30	0.31
21	0.10	0.125	42	0.32	0.305‡

* One reading. † Two readings throughout this column except where the star (*) and double dagger (‡) occur.

If the Bertrand method is considered as the more accurate it is seen that the Bang readings were not correct. With two exceptions, however, the average Bang reading at any of the sugar concentrations encountered was within 0.03 per 100 c.c. of blood of the Bertrand. This, of course, makes the actual percentage of error large. But as the essential feature of blood sugar determinations is not so much the absolute accuracy of the figures encountered—since objections to any one method can be found—as it is the relative value in a series of comparative determinations, the Bang method is perfectly satisfactory. This is illustrated by the accompanying chart made from the average of all readings encountered at all the different concentrations.

While the curve is not absolutely parallel, yet for comparative studies it is sufficiently similar. Furthermore, as control determinations were not made by the Bertrand method, it is possible that the differences occurring may have been considerably less.

Since his first publication, Bang[35] has modified the method slightly. The copper solution is made up as before, but 1 c.c. is used instead of 3 c.c. The protein is precipitated by hydrochloric acid in potassium chlorid instead of by acetic acid. For this purpose 1,360 c.c. of saturated potassium chlorid are diluted with 640 c.c. of distilled water and



1.5 c.c. of 25 per cent. hydrochloric acid is added. Of this mixture 6.5 c.c. are used for the precipitation and added boiling hot; 6.5 c.c. are added to wash the solution into the boiling flask. Titration is done with two-hundredths normal iodin made by diluting the original

35. Bang: Biochem. Ztschr., 1913, lvii, 300.

iodin solution to 100 c.c. instead of 50. The reagent is added until a permanent blue color appears. The amount of iodin solution used minus 12 divided by 4 gives the sugar values. In a few comparisons with Fehling's and Knapp's titration methods these results were found to be from 0.01 to 0.15 per cent. higher. This agrees with the results of Hirsch and Reinbach and Thannhauser and Pfitzer. Therefore to obtain most accurate results, Bang advises the subtraction of 0.015 from the estimated reading.

These modifications were used in a series of ten more bloods. In my experience, the results were no more accurate than by the first series. Furthermore so sharp an end-point in titration could not be obtained by the diluted iodin solution and therefore the former method is on the whole more satisfactory.

CONCLUSION

These comparative experiments show that the results obtained by the micromethod were not absolutely accurate if the results obtained by the Bertrand method are considered correct. In a series of forty-two determinations by both methods, however, in only two cases was there a difference of more than 0.03 gm. per one hundred c.c. of blood. This means that, while the absolute error was large, the relative error was slight. The curve of blood sugar determinations made from all the examinations was closely parallel. Hence for comparative and repeated studies in blood sugar estimations the micromethod is admirable. Furthermore, since the amount of blood required to make the test is so small, and the technic so simple, the method affords an excellent clinical means for the estimation of blood-sugar.

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BOOK REVIEWS

DISEASES OF THE STOMACH, INCLUDING DIETETIC AND MEDICINAL TREATMENT. By George Roe Lockwood, M.D., Professor of Clinical Medicine in the Columbia University; Attending Physician, Bellevue Hospital, New York. Pp. 624. Philadelphia and New York: Lea & Febiger, 1913.

In the preface of this book, the author states his intention, to "describe the diseases of the stomach as he has happened to see them." From this viewpoint the book is thoroughly satisfactory and deserving of hearty commendation. Moreover, such a presentation possesses a flavor of individuality and originality most welcome in an age of somewhat hackneyed disease description.

There are, however, inherent criticisms in such an endeavor applicable here as elsewhere. For the author in giving his own individual experiences fails to give critical consideration to the views of other workers in the same field, thereby omitting valuable clinical and experimental studies. This failure to include recent literature, while confessedly foreign to the author's intention, still detracts from the permanency of the book as an authoritative presentation of the subject. In other words, one misses the judicial impersonal weighing of the many problems in gastric disease, "the last word" feeling so impressively felt in standard German texts, so frequently wanting in our American. It might have been wiser had the available material, a "series of cases," been condensed and presented as a small monograph, in which form a complete survey of the field is neither expected nor desired.

The book includes instructive descriptions of gastric and duodenal ulcer (which, however, should *not* be considered together, as their pathogenesis in all probability is quite different); carcinoma (where unfortunately there *is* considerable doubt clinically and immeasureably more doubt pathologically and experimentally that they do arise from preceding ulcers); tuberculosis and syphilis, pyloric spasm, gastroptosis, neuroses, etc.

An interesting chapter on "Various Diseases and Their Gastric Relations," gives a thorough discussion of the appendix problem, and a thoroughly inadequate one of liver and gall-bladder disease; also a striking absence of reference to the relationship of diseases of women and the menstrual function; diseases of heart, kidney and metabolism; the anemias and leukemias, while chronic lead poisoning and the visceral crisis in erythemas are included. This lack of completeness, also evident in other chapters, is the most serious defect of the book.

Throughout, one feels, in the chapters concerning the more usual forms of stomach disease, the broad experience of the writer and is grateful for his conservative reasoning and sane clinical judgment. Likewise one must speak highly of the excellent plates of pathological specimens and the instructive radiograms. Also the graphic pain charts are most ingenious and refreshing and lend themselves well to class demonstration.

Several text errors have crept into an otherwise clearly typed and printed book. In the contents heading, Chapter X, "Anatomy of the Stomach," should probably be "Atony of the Stomach." On page 591, "The pancreatic fermentation may be demonstrated" for "The pancreatic ferments, etc." One would like to suggest also, that illustrative case histories be put in small type as they are elsewhere.

THE FUNDAMENTAL BASIS OF NUTRITION. By Graham Lusk, Professor of Physiology, Cornell University Medical College. Cloth. Price, fifty cents. Pp. 62. New Haven: Yale University Press, 1914.

On so vast a subject as nutrition it takes a great man to write a small book. Most of the controversial questions in nutrition are touched on in this little book of 51 pages, yet without bewildering the general reader with too much

technicality and discussion. Professor Lusk frankly recognizes the importance of the difference in the diets of different races and different climates. The account of the diet of Eskimos is especially interesting. The author notes that "although these people are the greatest meat eaters in the world, there is practically no gout among them. Also there is no quarreling. If two persons do not like each other, they simply move away from one another, but a blow is never struck." This point is made, no doubt, in answer to the claim so often made by vegetarians that meat stimulates the more quarrelsome side of our natures. Though aware that a man may live for long periods on low protein diet, Professor Lusk agrees with Rubner and Meltzer that the high protein requirement of Voit's standard ration is valuable as a "factor of safety" and of reserve in human life. The author's view of the Texan cow puncher's diet (page 33) is not made quite clear. There may be some misprint in this paragraph.

The author makes it clear that the American prejudice against veal has no basis in science. "In Germany light colored meats, such as chicken and veal, are considered digestible and good food for the sick. Red meat, like beef, is deemed indigestible. Veal is well cooked in Germany and greatly liked. The cooking of beef, on the contrary, is one of their unsolved problems. Ultimately the question is one of flavor. Man chooses what he likes best and, taken in moderation, what he likes best, he digests best."

The chapter on beri-beri and Funk's vitamines is especially interesting. Although clearly recognizing that polished rice is a dangerous diet, when other foods are taken in small quantities, as for example in Japan and the Philippines, the author believes that any legislation against polished rice in the United States is meddlesome and unnecessary. "The advocacy of unnecessary legislation weakens the authority of the medical profesion on sanitary matters."

In his final chapter on the monetary values of foods, the author makes a strong plea that some national authority should mark the food values on the packages in which food is sold. The manufacturer, he thinks, should be compelled by law to label his can: "This can contains x calories of which y per cent. are in proteins of grade C."

"If, through the mediums of the schools and the press everyone knew that a man of sedentary occupation required 2,500 calories and a laboring man 3,000 calories and more, no one suffering from want would spend his money for a can of tomatoes, which is little else than flavored water."

At the close of the book it is said: "Food manufacturers have recently requested a group of scientific men to meet as a board, name their own salaries and give advice. But the day of the sale of a man's scientific reputation and that of the institution with which he is connected has almost passed. The scientist has come to have sufficient altruism to believe that his services belong to all the people and not to a set of money making individuals."